Myrmecia pilosula complex (Hymenoptera: Formicidae) (Jack Jumper ant): Distribution, colony activity and behaviour

by

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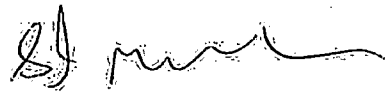

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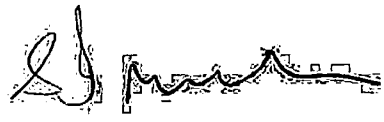
Signed 

Abstract

The Jack Jumper ant (*Myrmecia pilosula* complex) is limited geographically to the southern parts of Australia. It has a notorious reputation with the public of Tasmania because of the high incidence of allergic reactions to its sting in this part of Australia. The ant's aggressive attack and defence behaviour increases its exposure to the human population which is exacerbated because of its habit of nesting in close proximity to urban areas. Even though this ant has a very high public profile compared to other insects, very little research has examined aspects of *M. pilosula* that influence its exposure to the human population.

In this thesis I have addressed gaps in our knowledge related to *M. pilosula*. I have mapped their distribution, described the climatic envelope of their range and predicted their current distribution using climate parameters. On a local scale I have identified daily above ground colony activity patterns and within this, common behaviours displayed. Activity levels were not necessarily related to time of day, but did have a slight relationship with solar radiation. The exact relationship between solar radiation and colony activity has not been resolved. Other measured climatic conditions were found not to be related to ant activity. Particular behaviours displayed by *M. pilosula* throughout a day will increase exposure to a sting event at certain times of the day. *M. pilosula* was found to be associated with particular plant types which are used for foraging, in addition to associations between invertebrate abundance and presence of *M. pilosula* whereby significantly more invertebrates were found on plants with *M. pilosula*. The types of prey recovered at the nest were not reflected in arboreal samples of invertebrates associated with *M. pilosula*.

Activity at the nest surface decreases during the middle of the day. Humans are more likely to be stung at a nest in the mornings as the ants are exiting to forage, and in the afternoon when activity increases again after a lull in the middle of the day. To avoid foraging ants, humans partaking in outdoor activities should utilise the time early in the morning before the ants begin to exit the nest and late evening when most of the ants have returned to the nest. Any outdoor activities that have to be conducted near a nest surface should be considered during the middle of the day when activity at the nest surface is at its lowest. It is likely that *M. pilosula* forage

into the night therefore the risk of sting exposure after dark should not be discounted. Due to the arboreal nature of the ant, bushwalkers should remain on pathways when possible, avoid brushing overhanging foliage and in particular, that of *Eucalyptus* and *Acacia*.

Acknowledgments

Thank you to Dr Cas Vanderwoude, Brian Chung from Botanical Resources Australia and Dr Peter McQuillan for approaching me with the idea for this research. Associate Professor Simon Brown introduced me to the world of *Myrmecia* and allowed me to accompany him on multiple field trips to collect various species.

Peter McQuillan's support throughout this long process will always be remembered and appreciated. Thanks also to Dr Steve Leonard for agreeing to be a co-supervisor at such a late stage.

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My ability to complete this thesis relied heavily on friends and family who supported my decision to return to Australia from Sri Lanka to make the necessary changes. Once in Tasmania, I was faced with an unexpected impediment to my progress, during this time my friends and family interstate continued to provide me with an amazing network of encouragement which made me determined to continue.

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Chapter 1 Introduction

1.1 Background

The 'Jack Jumper ant' *Myrmecia pilosula* F. Smith, 1858 (Formicidae: Myrmeciinae) is an aggressive ant widespread in southern and eastern Australia (Figure 1-1). The ant's common name is derived from their characteristic response to disturbance, which consists of swarming from the nest utilising short swift jumps to gain ground quickly. This method of locomotion is also used to take prey by surprise when individuals are foraging.

Figure 1-1 Worker of *M. pilosula*

Importantly, *M. pilosula* is one of a few ants with a well known common name, and is readily recognisable to the Australian community in the southern states. It has gained this high profile because of its reputation as a dangerous ant due to its painful sting and relatively high incidence of allergic reactions to its venom.

Although most pest ants have the potential to have wide-ranging economic impacts, only a few species are well known for being a potential health hazard on a local scale (Table 1-1). *M. pilosula* is an exception: in Tasmania most people are well aware of the dangers of being stung by this ant, which is believed to have been the cause of five deaths between 1980 and 1999, exceeding deaths due to snake bite. Four of these deaths occurred in southern Tasmania and all of the victims had prior histories of *M. pilosula* or other *Myrmecia* venom allergy (Brown 2001; McGain and Winkel 2002).

Over an eight year period *M. pilosula*-related sting events caused between 21% and 25% of the 324 cases of anaphylaxis treated with adrenaline in the Royal Hobart Hospital Emergency Department, in Tasmania (Brown 2001). Anaphylaxis from *M. pilosula* venom is twice as common in Tasmania as that of the honeybee (*Apis mellifera*). As a result, epipens® (to administer adrenaline) are commonly carried by those who have an allergy to jack jumper venom, and an immunotherapy program is being developed for those with significant allergies (Wiese *et al.* 2008). Due to its notoriety, *M. pilosula* is treated as a significant pest ant in Tasmania and petrol or insecticides are often employed to destroy nests on private property.

Ant genus	**Country**	**Reference**
Pogonomyrmex	United States of America	(Pinnas *et al.* 1977)
Pachycondyla	Korea	(Cho 2002)
Myrmecia	Australia	(Brown 2003c; McGain and Winkel 2002)
Solenopsis	Australia United States of America	(deShazo and Soto-Aguilar 1993; Solley *et al.* 2002)
Cataglyphis	Egypt	(Sanad *et al.* 2002)
Polyrhachis	Thailand	(Wongsathuaythong *et al.* 1977)
Tetraponera	Thailand	(Wongsathuaythong *et al.* 1977)

Table 1-1 List of ant genera known to include species which have induced venom allergies and country where events occurred.

It is likely that the somewhat opportunistic nesting habits of *M. pilosula* promote exposure to humans. It has been observed that *M. pilosula* maintain nests in high abundance along vehicle and walking tracks (S. Brown, personal communication; personal observation). Vehicular transport along these conduits may aid the re-location of *M. pilosula.* The disturbance associated with road edges and tracks may provide preferred microclimatic conditions (such as increased insolation), and reduce ant diversity (and therefore competition) providing a greater opportunity for nest establishment (Gibb and Hochuli 2003). Some domestic backyards may provide an opportunity for the establishment of *M. pilosula* nests if they provide a favourable habitat, and are in close proximity to bushland where established source populations are present.

Because of the risks associated with being exposed to *M. pilosula* stings, methods to prevent interactions with the ant should be considered. The use of baits or pesticides

to treat nests is unsuitable due to the ant's endemism and the disruption this might have on local ecological processes. In addition, the ant is not a known threat to the ecology, trade or the economy and has no record of being transferred and establishing out of its native range. Due to the limited options for control, it is the responsibility of the populace to use knowledge of the ant to minimise interactions. Consequently it is important that research is conducted to gather further quantitative data about the biology and ecology of *M. pilosula*. This information is important for the local community so that they can reduce the likelihood of sting exposure, and for the scientific community to establish a more extensive knowledge base of this ant.

The range of ecological functions of species in the genus *Myrmecia* are not well known. Studying the behaviour of these ants is not appealing for many because of the ant's aggressive and dangerous nature. Gray (1974) suggests this may be the reason why colony populations have not been studied closely despite their scientific interest. In addition, their role in the broader invertebrate community is not well documented possibly as a result of a low capture rate of *Myrmecia* in biodiversity sampling compared to more abundant ant taxa (Andersen 2004; Hoffmann and Andersen 2003; King *et al.* 1998; Majer 1983), resulting in a research bias favouring dominant taxa, such as *Iridomyrmex* ants, some of whose behavioural and ecological interactions have been well studied (Davidson 1998; Ettershank and Ettershank 1982; Fox 1985; Gibb 2003; Greenslade 1987; Haering and Fox 1987).

1.2 Aims and objectives

Even though a venom immunotherapy program to manage venom allergies is a good prospect for the Tasmanian community, managing the risk of a sting event should also be considered a worthwhile and achievable goal. This thesis endeavours to determine what biotic and abiotic factors are particularly associated with *M. pilosula* in their native range, and how this information can be used by the local residents and the scientific community. It focuses on distribution and associated climate factors; daily colony activity and climate factors; arboreal food resources (prey type and vegetation); and individual ant behaviour.

The primary aim of this thesis is to identify parameters that affect:

1) The geographic distribution of *M. pilosula*,

2) Daily above ground colony activity of *M. pilosula*,

4) Potential and actual resource use, and

3) Specific behaviours of individual *M. pilosula*.

Within this framework the specific objectives are to:

- Map known locations of *M. pilosula*.
- Identify what climate conditions best describe the climatic envelope of locations where *M. pilosula* have been collected.
- Model the predicted distribution of *M. pilosula* using climate layers.
- Identify daily activity patterns and classify common behaviours displayed by *M. pilosula*.
- Isolate variables that might drive daily activity cycles of *M. pilosula*.
- Identify associations between presence of *M. pilosula*, foraging habitats and potential prey.
- Determine how this knowledge can be used to minimise interactions with the human community and add to the body of scientific knowledge related to this high profile ant.

This thesis contains an abstract, introductory chapter, literature review, materials and methods chapter, three research chapters, discussion and concluding chapters. Chapter 1 gives an introduction to the research topics and outlines the research aims and objectives. Chapter 2 reviews literature relevant to *Myrmecia* while outlining previous and current research interests related to *M. pilosula*. Chapter 3 describes the general materials and methods used in the subsequent experimental chapters. Chapter 4 describes the environmental conditions that *M. pilosula* inhabits and its predicted current distribution. Chapter 5 is a study of the activity of a number of *M. pilosula* nests at a selected locality in Hobart and arboreal food resources. Chapter

6 presents comprehensive observations on a selected *M. pilosula* colony over one day and explores the relationship between activity, behaviour and small scale climate conditions. Chapter 7 synthesises the information presented in previous chapters and offers suggestions on how the information presented can be used in the management of *M. pilosula* and suggests potential future research objectives.

The scope of this thesis encompasses the biology and ecology of the *M. pilosula* species complex. The taxonomy and nomenclature of this species complex is controversial and still being formalised (Taylor pers.comm.). In general, the use of the term *M. pilosula* in this thesis refers to the entire species complex, unless otherwise stated. The local Hobart populations of *M. pilosula* such as those studied in Chapters 5 and 6 are very likely to be *M. pilosula* species itself (Taylor, pers. comm.), especially since Hobart is the type locality for *M. pilosula* (s.s.). However as the species was not able to be confirmed for this study, voucher specimens have been lodged in the entomology collection, at the School of Geography and Environmental studies at UTAS, for future reference.

Chapter 2 Review of literature related to the ant genus *Myrmecia* (Hymenoptera: Formicidae: Myrmeciinae)

2.1 Introduction

Ants of the genus *Myrmecia* are iconic Australian fauna and have attracted considerable scientific interest over two centuries. Their large size, fierce appearance and widespread distribution over the continent means they are familiar to most people and they have attracted a variety of vernacular names, including bulldog ants, inchmen and jack jumpers. Several Australian sporting clubs use bulldog ants as their mascot, channelling their fighting spirit.

Public awareness of *Myrmecia* is exceptional in that it is one of few native ant taxa that have widely recognisable common names. Most *Myrmecia* are broadly referred to as bull ants or inch-men. Other well known native ants include the green headed ant, *Rhytidoponera metallica* (South East Queensland), green tree ant *Oecophylla smaragdina* (Northern Australia) and meat ants *Iridomyrmex purpureus* (South Eastern Queensland). These common names are often misused: for example, the name green headed ant is often given to all *Rhytidoponera* spp. while both *Rhytidoponera* spp. and *Oecophylla smaragdina* are referred to as green ants depending on the region, and the name meat ant is generally associated with all *Iridomyrmex* (Andersen 2002). An exception to this confusion is the species complex *Myrmecia pilosula*. Although limited to Southern Australia, it is well recognised as the jack jumper or jumping jack ant. Even though some other species of *Myrmecia* have a similar jumping behaviour (e.g. *Myrmecia nigrocincta*), it is only *M. pilosula* that has its common name firmly established.

However, it is interesting to note that, even though *Myrmecia* and, specifically, *M. pilosula*, have high public profiles locally, there is limited information on their interactions with other ants and their habitats. This is demonstrated by a search of the ant literature data base FORMIS 2009 (Wojcik and Porter 2009) using *Myrmecia* as a key word which retrieved 479 publications (slightly over 1%). Only 29 publications had *Myrmecia pilosula* in the title of the paper and of these 23 had venom, allergy or chromosome as keywords. Of the remaining 6 articles, only 3

were related to *M. pilosula* interactions with their surroundings. To a degree, this lack of information is a consequence of the limited global range and isolation of *Myrmecia*. Even so, this ant has some characteristics that make it unique and worthy of further research outside of the currently limited scope. The following literature review covers aspects of *Myrmecia* biology and behaviour that have interested researchers in the past, with attention given to aspects of *M. pilosula* which make it distinctive.

2.2 *Discovery and taxonomy*

The taxonomy of *Myrmecia* dates back to 1775 when Fabricius described *Myrmecia gulosa* from specimens collected at Botany Bay on Cook's first expedition to Australia in 1770. Further descriptions of new species accumulated through the nineteenth century, peaking before 1950 then declining to the current low rate of new names being proposed (Figure 2-1). A comprehensive historical review of *Myrmecia* taxonomy is given by Ogata and Taylor (1991).

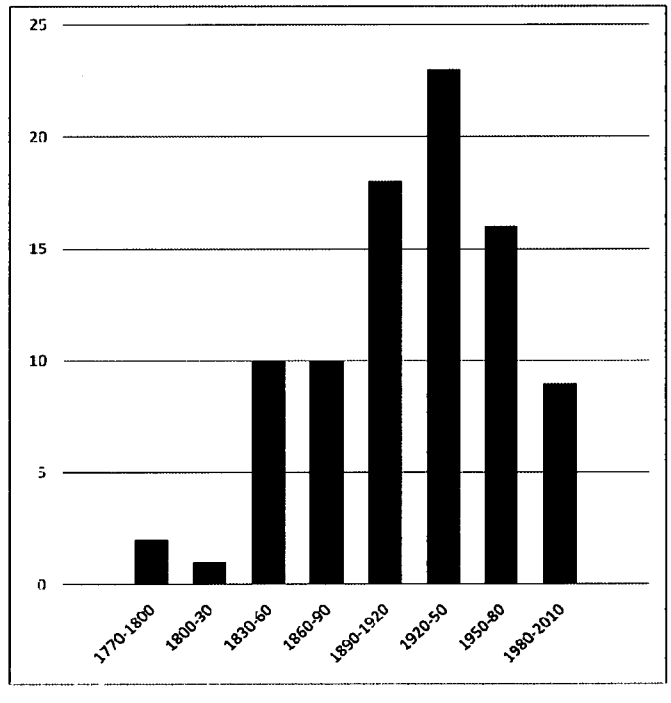


Figure 2-1 Descriptions of new Australian *Myrmecia* species at 30-year intervals

2.3 Phylogeny

Phylogenetic and fossil studies indicate that the Myrmeciine ants are a formerly widespread group that, by the late tertiary, became extinct everywhere except in the Australian region (Haskins and Haskins 1950; Ward and Brady 2003). The subfamily Myrmeciinae contains two monotypic tribes, Myrmeciini and Prionomyrmecini (Ward and Brady 2003). They are of great interest due to their archaic heritage and may represent the most basal group of living ants (Haskins and Haskins 1950; Wheeler 1932). The tribe Myrmeciini contains the single genus *Myrmecia* while *Nothomyrmecia* is the sole living genus representing the Prionomyrmecini (Ogata and Taylor 1991; Taylor 1978). However, despite the antiquity of the subfamily Myrmeciinae, it has been concluded that the genus *Myrmecia* itself may be relatively young (Hasegawa and Crozier 2006). *Myrmecia* comprises nine species groups, which can be morphologically split into two phylogenetic groups on the basis of possessing or lacking an occipital carina. The evolutionary history of *Myrmecia* has been inferred by Hasegawa and Crozier (2006), although they acknowledge that there are still some unresolved paradoxes in their results that need to be explored.

The genus *Myrmecia* contains the *pilosula* group which comprises 15 species (Ogata and Taylor 1991), including the *pilosula* species complex which is karyotypically diverse although minimally varied morphologically (Crosland *et al.* 1988; Crozier 1995). *Myrmecia croslandi* is the most karyotypically divergent species within this complex (Crozier 1995) and unique in that it has a chromosome number of n=1, which only one other multicellular organism is known to share (Crosland and Crozier 1986). Subsequently "*M. pilosula* n=1" was elevated to species rank as *Myrmecia croslandi* by Taylor (1991). Phylogenetic analysis shows that *M. croslandi* is actually a sister taxon to the rest of the species in the *pilosula* complex (Crozier 1995; Taylor 1991). In total there are seven recognised species within the *M. pilosula* species complex, five of which are undescribed at present (Taylor pers. comm.). The alpha taxonomy of the *M. pilosula* species complex is currently under review. A manuscript in preparation also describes the biogeographical distribution

of each species and previously unreported morphological differences (Taylor pers. comm.). However, at the moment it is not possible to confidently separate the species, consequently only references *M. pilosula* complex are made hereafter.

2.4 Past research

Myrmecia ants tend to be under represented in general studies on ant ecology in Australia (Hoffmann and Andersen 2003). This is due to their low population density, lack of dominance, low 'catchability'(Andersen 1983; Hoffmann and Andersen 2003) (Figure 2-2), and possibly as a result of the biases associated with pitfall trap methods commonly used to census ants (Abensperg-Traun and Steven 1995; Schlick-Steiner *et al.* 2006). Therefore their ecological function has not been examined as closely as that of more abundant ant taxa in current research.

Figure 2-2 *Myrmecia* sp. with brood at edge of pitfall trap (circled). This ant backed up very slowly and managed to avoid falling in.

Much literature related to Myrmeciinae ants focuses specifically on their biology or 'social structure' (Clark 1925a; 1925b; 1927; Haskins and Whelden 1954; Wheeler 1932), rather than their ecological role. Many studies have been conducted on the phylogeny and genetics of *Myrmecia* (Hasegawa and Crozier 2006; Ward and Brady 2003). Interest in *M. pilosula* has further developed due to its unusual chromosome numbers (Crosland and Crozier 1986) and venom composition (Billen 1990; Donovan *et al.* 1993; Matuszek *et al.* 1992). Current research focuses on biomedical techniques related to venom immunotherapy (Brown and Alewood 2001; Brown 2003b; c; Wiese *et al.* 2006). Biomedical research focus has also included biochemical analysis related to the allergenic importance of proteins in the venom of

M. pilosula (Davies *et al.* 2004; Donovan *et al.* 1993; Matuszek *et al.* 1994; Wiese *et al.* 2006). Although some aspects of the biology of a small number of *Myrmecia* species have been studied, no studies have been devoted to the distribution, above ground daily nest activity or the individual ant's behavioural activity of *M. pilosula.*

2.5 Ecology

Ecologically, *Myrmecia* are classified as Specialist Predators (Andersen 1995). These are described as 'medium to large sized ant species which predate on other arthropods, and have little competitive interaction with other ants due to their specialized diets and typically low population densities' (Andersen 1995).

Many species of *Myrmecia* are sympatric in their distribution, but the actual mechanisms regulating competition for resources between *Myrmecia* species in the field are not well known. Muir (1974) examined ecological traits of 4 sympatric *Myrmecia*, finding that resources were shared and foraging times overlapped, and surmised that this sharing of resources may limit the abundance of each species. Colonies also attacked and destroyed founding colonies of the same species in 'close' proximity to an established nest. Aggression between species also occurred but in a different context and only if contact was frequent i.e. many individuals of different species foraging at the same time.

Crosland (1989) undertook a series of laboratory investigations into aggression in *Myrmecia* and discovered that intra-colony aggression does occur, although it is not known whether the laboratory environment may have affected the ant's behaviour. It has been suggested that the displayed aggression may have been due to competition amongst workers to lay eggs (Crosland 1989). Crosland (1989) found that inter-colony aggression also occurs but is shown to be greater between allospecifics than conspecific worker ants. Competitive mechanisms of other ants have been studied but these have mostly focused on invasive ants and their impact on a native ecosystem (Holway 1999; Human and Gordon 1999; LeBrun *et al.* 2007; Morrison 2000).

2.6 Nesting habits

Myrmecia nests have been observed in a broad range of habitats (Dietemann 2002; Freeland 1958; Greenslade and Thompson 1981; Haskins and Haskins 1955; Higashi and Peeters 1990). Preferred nesting sites differ between species of *Myrmecia* and include under trees, in open spaces, in moist soil or under logs (Gray 1974; Haskins and Haskins 1955). However a general preference for woodland habitats over grassland (Muir 1974) is exhibited.

In the initial stages of founding a colony, the internal nest structure is very similar among *Myrmecia* species, although there is some variation in established nests (Gray 1974) which may limit colony size (Gray 1971a). Although most species nest in the soil, some also nest among rocks or in rotting logs and stumps (Lowery 1994). Nests of small sized *Myrmecia* have smaller mounds which are often camouflaged by leaf litter, debris and grass, or may have no mound. The small *M. urens* nests in the soil and after rain the nest is sometimes surmounted with an earthen spout 1-3 cm high (Lowery 1994). Larger ants such as *M. brevinoda* build a nest with a distinct mound, complex series of shafts and one opening that is used more frequently than others (Gray 1974; Higashi and Peeters 1990). In this species nests may house up to 2576 workers (Higashi and Peeters 1990). In contrast, nests of *M. dispar* colonies are difficult to locate as a result of their camouflaged nests that involve limited excavation (Gray 1971b). For a more detailed description of a *Myrmecia* nest structure including size of tunnels and chamber use see Gray (1971a).

M. pilosula nests are characteristically low mounds decorated with gravel and leaves. Nests occur frequently in light bush or dry sandy soils (Clarke 1986) but they have also been observed infrequently in soil with embedded large rocks or under mounds of moss (personal observations). Sorted gravel particles of similar size are used on top of ant nests to absorb raindrop impact which is believed to aid nest longevity (Cowan 1985). It is believed that rocks are used to protect colony members against extreme temperatures and serve as a supplementary source of heat (Janson *et al.* 2002; Thomas 2002). In urban areas *M. pilosula* may gain the same protection and source of heat from nesting nearer to buildings or underneath pavements or driveways (e.g. (Kemp *et al.* 2000)). It is likely that moss cover on nests occurred

subsequent to nest establishment and may indicate nest longevity (R. Patterson, personal communication).

2.7 Colony dynamics

Most *Myrmecia* are monogynous although some such as *M. pilosula* exhibit colony founding by pleometrosis (Crosland 1988; Haskins and Haskins 1950). After mating, some species of *Myrmecia* found their nests through partially-claustral habitation (Haskins and Haskins 1950; 1955; Wheeler 1932). This type of colony founding is considered to be a primitive behaviour that has not evolved to the fully-claustral founding displayed by higher ant genera.

Wheeler (1932) first described feeding in *Myrmecia* and reported that the larvae are fed with pieces of freshly killed insects while adults fed exclusively on nectar and sap. These observations are supported by Haskins and Haskins (1950). Since then, further observations on *Myrmecia* have determined that feeding methods differ among species. Workers of some species specialise in either egg feeding (*M. gulosa)* or feeding by trophallaxis (*M. regularis* and *M. vindex*) possibly depending on the degree of ovarian development. Species with underdeveloped ovaries such as *M. brevinoda* may provide a more nutritious meal via trophallaxis in contrast to species with well developed ovaries such as *M. gulosa* (Crosland 1988). The workers of *M. gulosa* collect eggs from the tip of their gaster with their mandibles as they produce them and feed them directly to the larvae. *M. gulosa* workers also place larvae on an egg pile for the larvae to feed without any further assistance (Crosland 1988). The arboreal foraging nature of *M. pilosula* suggests a strong reliance on tree-based resources for food (personal observation).

In at least two species, *M. brevinoda* and *M. gulosa,* size polyethism occurs among workers. The larger workers hunt, defend and build the mound and the smaller workers excavate soil deeper in the nest (Dietemann 2002; Gray 1973; Higashi and Peeters 1990). This division of labour is thought to be common only in the larger species of *Myrmecia* (Gray 1974).

Foraging times may differ between species (Gray 1971b) on both a daily and seasonal basis. For example, daily foraging times differed in a community of four

sympatric species (Figure 2-3), and this was reflected in their eye structure (Greiner 2007). This segregated pattern of activity in these ants may help to avoid competition as discussed previously. *M. desertorum* colonies are more active foragers during the winter time with activity increasing as the day progresses, whereas *M. dispar* colonies are more active in the summer during early morning and late afternoon. *M. desertorum* workers were also more likely to forage into the night, unlike *M. dispar* workers (Gray 1971b).

2.8 Sting allergies

Sting allergy prevalence is determined by age and exposure rate. Sting events associated with *M. pilosula* are common because nesting sites and individual foragers are often in areas where interactions with humans are likely to occur. Sting exposure in Tasmania is excessive compared to mainland Australia, and there is a high systemic reaction risk in allergic people on re-sting (Brown 2003a). Exposure is common in both rural and urban areas of Tasmania, and nests are frequently encountered in back yards, farms and walking tracks (pathways in suburbia or bush tracks) (personal observations). The establishment of *M. pilosula* nests in areas of frequent use by the community is therefore a genuine health hazard.

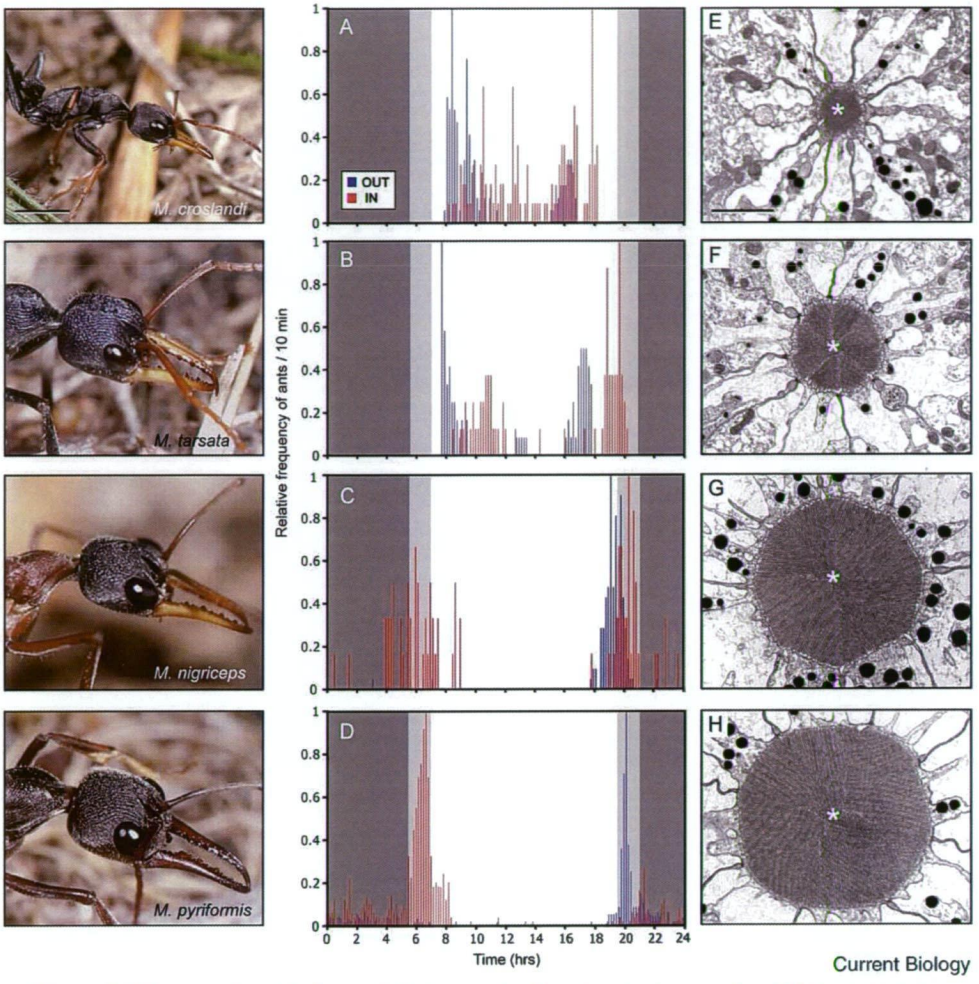


Figure 2-3 Foraging bout timing and photoreceptor diameters in four species of *Myrmecia* ants (left).(A–D) 24 hour activity plots showing outgoing (blue) and incoming (red) forager traffic at the nests of the four *Myrmecia* species (A) *M. croslandi*; (B) *M. tarsata*; (C) *M. nigriceps*; (D) *M. pyriformis* (Greiner 2007).

2.9 Biomedical techniques and immunotherapy

The allergenic properties of *M. pilosula* venom was first explored by Clarke (1986) and the first allergens were chemically identified by Ford and Baldo (1991). To assist development of a desensitising vaccine, further studies and identification of the major allergenic peptides (1 and 2) in *M. pilosula* venom have since been undertaken (Donovan *et al.* 1993; Street *et al.* 1996). An immunotherapy trial conducted by Brown *et al.* (2003c) concluded that venom immunotherapy had some potential to prevent deaths in the human population. Studies of the allergenic properties of *M.*

pilosula venom continued and major peptides 3, 4 and 5 were subsequently cloned and characterised (Inagaki *et al.* 2008; Inagaki *et al.* 2004; Wiese *et al.* 2006). The nomenclature used to describe *M. pilosula* venom has been revised and updated (Wiese *et al.* 2007). This information is now being used to validate a range of procedures used during venom immunotherapy trials, with the intention of standardising an allergy vaccine for the purpose of diagnosis and treatment rather than research (Wiese *et al.* 2008).

Further research into *M. pilosula* should focus on enabling the public to understand how to reduce the likelihood of coming into contact with the ant and therefore reducing the likelihood of a sting event. Learning more about the ant's distribution would help describe what continental conditions influence its spread. Increasing our knowledge of the ant's daily activity and behavioural habits will make us more aware of when the ant is active and what biotic (e.g. preferred foraging habitats) and abiotic (e.g. local weather) conditions drive its activity.

Chapter 3 Research design

3.1 Distribution of M. pilosula *in relation to climate*

To assess the climate envelope of *M. pilosula*, historical collections were used to map the known distribution of *M. pilosula* throughout Australia. A climate modelling package, BIOCLIM (Fischer 2001), was used to describe a number of climate parameters at locations where *M. pilosula* has been collected and MaxEnt (Phillips *et al.* 2006) was used to predict current distribution.

BIOCLIM has been identified as a useful tool to predict distribution using long-term average climatic conditions (Beaumont *et al.* 2005; Lindenmayer *et al.* 1991; Manning *et al.* 2005), determine distribution limits (Jumbam *et al.* 2008; Lindenmayer *et al.* 1991; Manning *et al.* 2005) and to describe climatic parameters in which particular species are located (Fischer 2001; Manning *et al.* 2005). General limitations of BIOCLIM are outlined in Nix (1986) and Doran and Olsen (2001).

BIOCLIM was not used as a predictive modeller as it required absence data which was not available. Instead, presence only data was used to predict current distribution using maximum entropy modelling within the computer package, MaxEnt (Phillips *et al.* 2006) and climate layers from WorldClim (http//www.worldclim.org) (Hijmans *et al.* 2005). MaxEnt produces a model of a species ' using occurrence locations and environmental layers. The computed model expresses a probability distribution over a set of grid cells in a landscape.

3.2 Location of study sites

Field studies of *M. pilosula* colonies were conducted near Hobart, Tasmania, in November 2009 and March 2010. Tasmania is an intermittent continental island off Southern Australia, encompassing 68 331 square km, with a cool temperate maritime climate (Table 3-1). The 200km wide Bass Strait separates Tasmania from the Australian mainland. Hobart is the southern-most state capital city in Australia and is located near the estuary of the Derwent River (Figure 3-1). Geologically, most of

the eastern half of Tasmania is formed of Jurassic dolerite. Western soils contain a mixture of Cambrian and Neoproterozoic minerals, while in the far North-East Devonian granites and Ordovician Mathinna beds dominate (Figure 3-2).

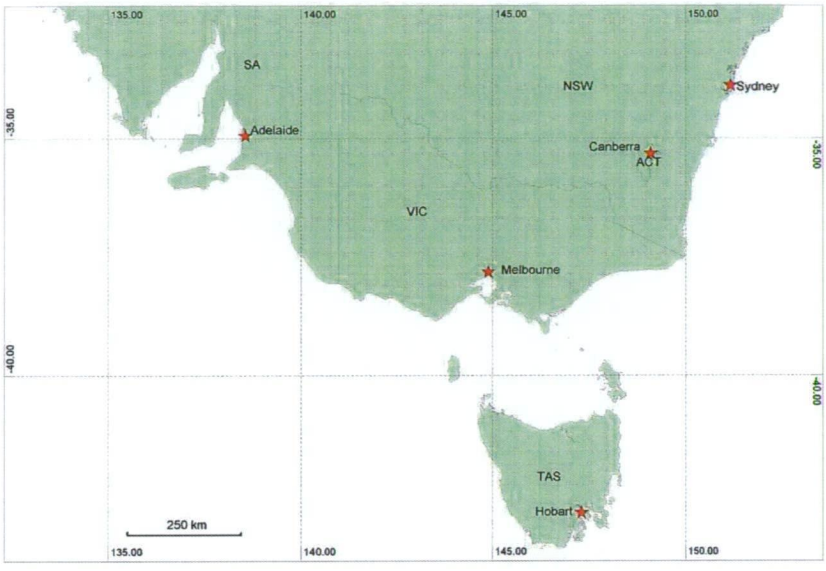


Figure 3-1 Map of Southern Australia showing location of the state of Tasmania and the state capital, Hobart (Map by Jon Marsden-Smedley).

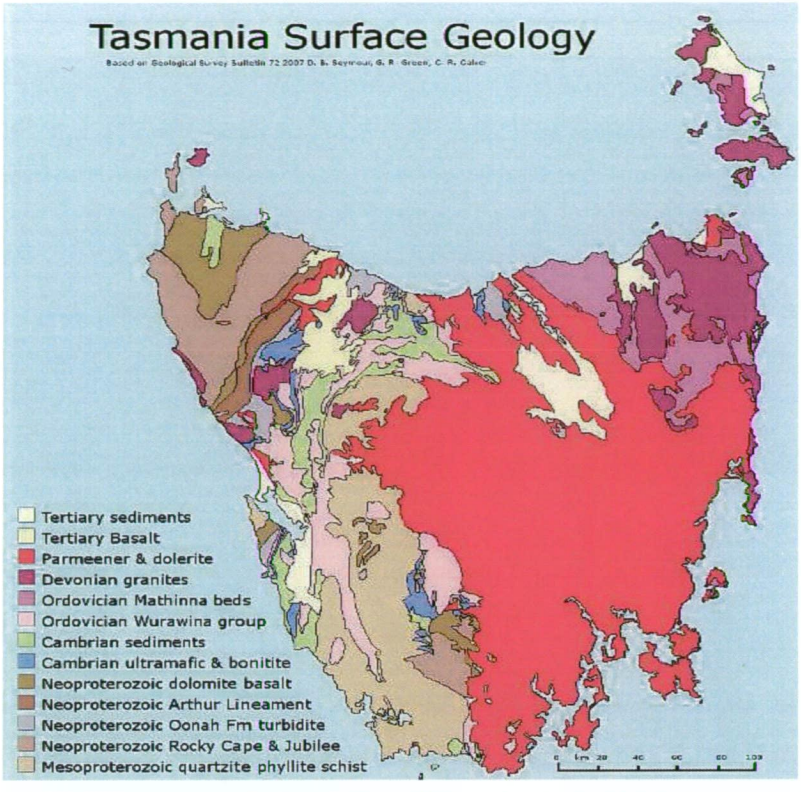


Figure 3-2 Surface geology of Tasmania (Bartlett 2008)

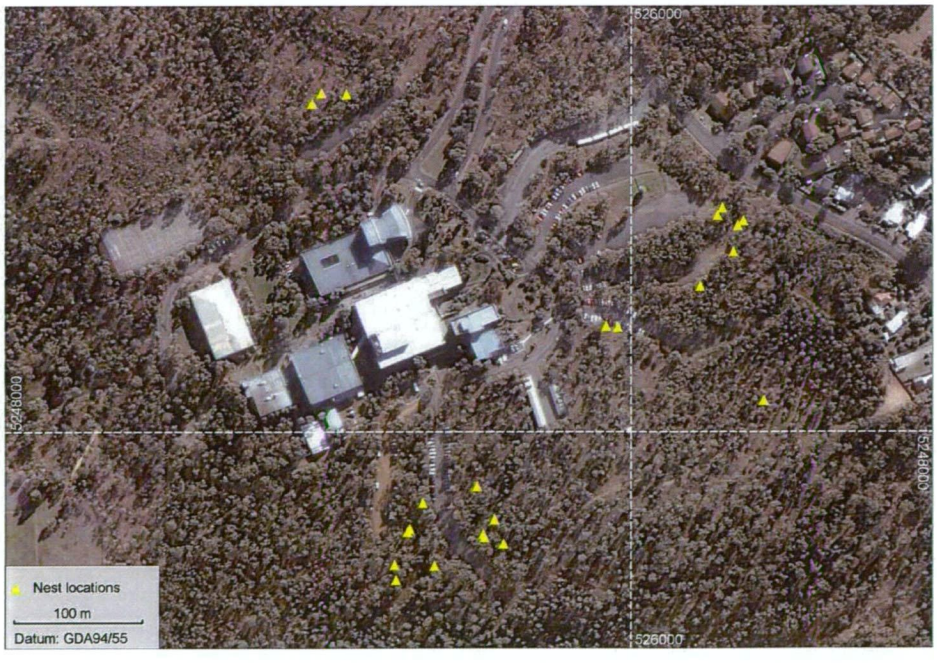


Figure 3-3 Aerial photograph of study site location, Hobart College campus, Mt Nelson. Colony locations are marked with yellow triangles (Photograph contributed by Jon Marsden-Smedley).

3.2.1 Colony locations

The colonies used for the study were located on Mt Nelson; a suburb located about 4km south of the Hobart CBD and approximately 300m above sea level. The study site surrounds the Hobart College campus, Olinda Grove and is accessible via the Southern Outlet from Hobart CBD (Figure 3-3). This site was partly chosen for its accessibility and proximity to the University of Tasmania, Hobart Campus.

The soil on Mt Nelson is a grey-brown podzolic on dolerite described as, 'imperfectly drained texture contrast soils developed on Jurassic dolerite bedrock and colluvium on rolling to steep (10-56%) land' (http//www.thelist.tas.gov.au). Vegetation is a mix of *Eucalyptus globulus* and *Eucalyptus pulchella* dry forest and woodland, in addition to small areas of *Eucalyptus obliqua* and lowland grassland complex (http//www.thelist.tas.gov.au). Table 3-1 summaries monthly climate records for Hobart.

A general survey for *M. pilosula* nests was conducted in different locations on Mt Nelson. Once it was confirmed that *M. pilosula* nests were established on Mt Nelson, a more targeted approach was used to identify individual nests. Three adjacent east/west linear transects 10 metres apart were established at 3 locations. Nest surveying took place by walking along each transect and scanning the ground for 5 metres on each side. Any feature that fitted the typical characteristics of a nest (Figure 3-4) was disturbed by inserting a metal rod repeatedly to incite a reaction so that any inhabitants could be collected and identified. Rocks and logs were dislodged to expose any nests that may have established underneath (Figure 3-4).

a) **b)**

Figure 3-4 a) Typical nest surface construction, slightly raised with a consistent surface covering of pebbles b) Nest construction between rocks, surface construction similar to a).

Statistics	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual	Years
Temperature														
Mean maximum temperature (°C)	22.1	22.0	20.2	17.7	15.1	12.4	12.2	13.7	15.4	17.2	18.9	20.4	17.3	29
Mean minimum temperature (°C)	12.6	12.6	11.3	9.4	7.6	5.3	4.9	5.7	6.9	8.3	9.9	11.3	8.8	29
Rainfall														
Mean rainfall (mm)	44.4	36.4	39.6	44.2	36.4	44.0	48.7	58.6	57.5	56.4	46.0	57.1	569.1	29
Decile 5 (median) rainfall (mm)	35.7	24.2	30.8	44.4	31.2	38.8	40.2	59.0	41.4	49.6	46.8	40.6	570.6	29
Mean number of days of rain ≥ 1 mm	5.7	5.0	6.4	7.0	6.3	7.2	8.6	9.5	9.8	9.6	7.5	7.8	90.4	29
Other daily elements														
Mean daily sunshine (hours)	7.9	7.8	6.5	5.6	4.5	4.2	4.5	5.3	6.1	7.1	7.4	7.4	6.2	15
Mean number of clear days	4.5	4.4	4.4	4.2	3.8	3.6	3.6	3.5	2.6	3.2	3.0	3.3	44.1	29
Mean number of cloudy days	14.5	12.1	14.3	13.6	15.6	14.1	14.0	14.6	13.9	14.9	15.9	15.8	173.3	29
9 am conditions														
Mean 9am temperature (°C)	16.2	15.8	14.3	12.6	10.1	7.4	7.2	8.5	10.7	12.2	13.7	15.2	12.0	29
Mean 9am relative humidity (%)	61	65	69	70	75	79	78	72	65	63	62	60	68	29
Mean 9am wind speed (km/h)	15.0	13.7	13.4	14.8	14.2	13.7	13.7	16.0	17.3	17.3	15.8	15.6	15.0	29
3 pm conditions														
Mean 3pm temperature (°C)	20.0	20.3	18.7	16.2	14.0	11.3	11.2	12.5	13.9	15.6	17.1	18.5	15.8	29
Mean 3pm relative humidity (%)	51	51	54	57	60	64	62	56	54	52	53	51	56	29
Mean 3pm wind speed (km/h)	21.3	19.8	18.2	16.8	15.2	14.6	15.1	17.4	19.5	20.6	20.7	21.8	18.4	29

Table 3-1 Summary of climate conditions for Hobart (data collected over 15-29 years) (www.bom.gov.au)

Nests were found in *Eucalyptus pulchella* grassy woodland, *Eucalyptus pulchella* grassy forest and *Themeda triandra* tussocky grassland. Nests were not found in *Eucalyptus globulus* grassy woodland, *Eucalyptus* open woodland nor in *Allocasuarina* forest (Figure 3-5).

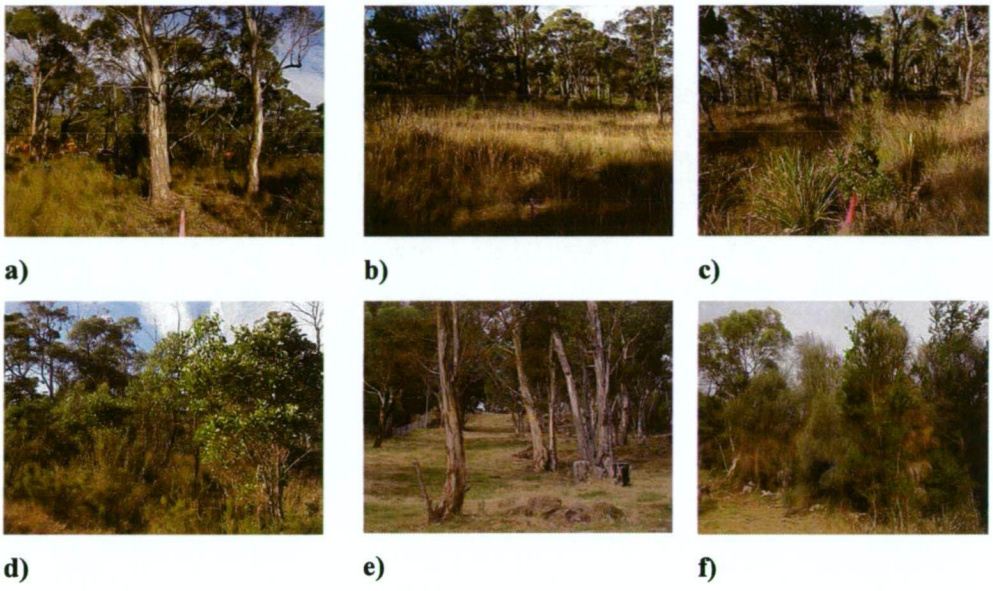


Figure 3-5 Vegetation types surveyed for *M. pilosula* nests on Mt Nelson. a) *Eucalyptus pulchella* grassy woodland b) *Eucalyptus pulchella* grassy forest c) *Themeda triandra* tussocky grassland d) *Eucalyptus globulus* grassy woodland e) *Eucalyptus* sp. open woodland f) *Allocasuarina* forest. *M. pilosula* were present in vegetation types a)-c) but absent in types d)-f).

Chapter 4 Distribution of *M. pilosula* in relation to climate

Abstract

The aims of this study were to i) generate a bioclimatic profile of *M. pilosula* using the program BIOCLIM and ii) to make a prediction of its current distribution using climate data from www.worldclim.com which was then run in the program MaxEnt using known location data. A bioclimatic profile was produced based on the location of 446 reliable samples of *M. pilosula* from the collections held by various institutions. Principle Component Analysis (PCA) was used to help identify key climate parameters most strongly related to *M. pilosula* locations. Scatter plots, correlation matrices and frequency histograms were used to identify a climate envelope and climate thresholds in relation to the known range of *M. pilosula*. The main factor limiting *M. pilosula* populations in Australia is high temperatures during the hottest time of the year. Other factors such as moisture and radiation are also important, but would need to be considered in conjunction with other parameters if used as a predictor of distribution. Predicated current distribution was within the climatic envelope identified by BIOCLIM although expanded on recorded occurrences and therefore identified possible gaps in collection records. For the purposes of this study MaxEnt could have been used in lieu of BIOCLIM in order to meet both aims; i.e. to describe the climatic envelope at known locations and for mapping predicted range.

4.1 Introduction

Myrmecia has been collected widely across southern regions of Australia with a greater density of records in the south-west (north to Geraldton to east of Esperance), in the south-east (encompassing most of eastern South Australia and New South Wales), and all of Tasmania and Victoria with distribution in Queensland mainly along the coastline. Within this range, many of the species are bioclimatically restricted, such as *M. desertorum* which is confined to the hot, dry interior of Australia, *M. arnoldi*, found only in the far south of Western Australia, and *M. brevinoda*, with a limited range on the east coast (Clark 1951; Shattuck 1999). Although these species are only a cross section of the genus, their distribution is an indication that some species within this species are influenced by bioclimatic pressures which constrain their range. It is likely therefore that the distribution of *M. pilosula* is similarly affected.

M. pilosula has a known geographic range that spans the temperate parts of southern Australia, with a concentration of records from the south eastern corner of the continent (Shattuck 1999). A few specimens have been collected in Western Australia and Queensland (Shattuck 1999). Current descriptions of the distribution and habitat are vague and generalised, e.g. 'sandy soiled highland areas of South-eastern Australia and Tasmania' (Donovan 1994) and '....beaches, in coastal scrub and heath, in dry sclerophyll, grasslands and lawns, ranges and the Central Plateau, and it has spread rapidly along the roadsides', but is not found '....in moss or rainforest nor in any form of forest with a thick understorey of bracken, blackberry or anything else' (Lowery 1994). A study of *M. pilosula* habitats in Hobart, Tasmania found that their presence was related to the occurrence of dry sclerophyll vegetation types (Evans 2008).

At present the only published geographic distribution of *M. pilosula* has been generated from samples held at the Australian National Insect Collection, CSIRO (Shattuck 1999). This collection, although extensive, does not include distribution data represented by specimens held by other institutions in Australia. The factors that influence its distribution in Australia have not been explored in depth. The Australian ant fauna is often described by the habitat type it occupies within a local

region (Andersen 1993; Andersen and Majer 2000; Andersen and Yen 1992) without consideration for which biogeographical characteristics influence its distribution on a larger scale. A focussed study is required to gain a better knowledge of what influences the presence of *M. pilosula* and therefore inform policies to enable better control over its interactions with human populations.

In addition, due to a lack of targeted surveys, the detailed distribution of *M. pilosula* is unknown. Indentifying the distribution of *M. pilosula* would open up opportunities for a more targeted approach to sampling and enable the development of focussed research into what drives the distribution of *M. pilosula*, and how this affects interactions with the human population. As discussed, *Myrmecia* populations in Australia appear to have defined ranges which they occupy. This study aims to identify which climate conditions best describe the climatic envelope within which *M. pilosula* occurs, and to predict current distribution of *M. pilosula* using known location data.

4.2 Methods

Collection records for *M. pilosula* were gathered from a range of institutions and individuals (Table 4-1). According to Taylor (pers. comm.) collections of *M. pilosula* exist all over the world with at least 23 institutions holding specimens. For the purposes of this study only local institutions were contacted for collection information, with the exception of the Natural History Museum, London. This data set is believed to be representative of *M. pilosula* populations over its full range (Shattuck 1999).

Only records with complete location information were used and duplicates were discarded. Records only identified to "*M. pilosula* species group" were also discarded, the intention being to focus specifically on the taxa most closely related to the species found in Tasmania. Consequently, records from the Australian Museum and some from the ANIC collection were not used. All remaining specimens have been identified by Dr R. Taylor.

Latitude, longitude and location description were collated from the records for each specimen. Co-ordinates in degrees, minutes and seconds were converted to decimal degrees by using the following formula:

Decimal degrees = degrees+(minutes/60)+(seconds/3600)

Each co-ordinate was calculated and the elevation in metres of each co-ordinate was assigned using the web page GPS Visualizer (www.gpsvisulizer.com).

Climate factors for each co-ordinate were generated by the computer package BIOCLIM, a bioclimatic prediction system which uses surrogate terms (bioclimatic parameters) derived from mean monthly climate estimates, to approximate energy and water balances at a given location (Nix 1986). BIOCLIM produces values for 35 bioclimatic parameters based on climate surfaces utilising the variables "maximum temperature", "minimum temperature", "rainfall", "solar radiation" and "pan evaporation" to give a value for each parameter at each co-ordinate.

Principle Component Analysis (PCA) was used to simplify the BIOCLIM parameters by determining which load most highly on the fewer axes that best explain the climatic gradients along which the ants are located. The first two PCA axes which were most influential were retained, and the parameters which had an Eigenvector value of greater than (+/-) 0.2 were graphed in a scatter plot and entered in a correlation matrix. PCA was performed with the statistics program JMP (www.jmp.com). Plots of frequency distributions were used to help determine climatic thresholds.

As BIOCLIM was limited to generating climate conditions of known locations, the computer program MaxEnt (Phillips *et al.* 2006) was used to map predicted current distributions, defined by climate conditions generated by Worldclim (www.worldclim.com). Worldclim is a set of global climate layers (climate grids) with a spatial resolution of one square kilometre.

Collection	Contact person	Records accessed by	Institution holding collection
CSIRO ANIC	Dr Steve Shattuck	www.anic.ento.csiro.au/ants/biota_details.aspx?BiotaID=37534	CSIRO
CSIRO ANIC	Dr Bob Taylor	Personal communication	CSIRO
Australian Ant Venom Allergy Study	Professor Simon Brown	Personal communication	Royal Hobart Hospital, Tasmania
Australian Museum, Entomology	Dr Dave Britton	Personal communication	Australian Museum, Sydney
B.B. Lowery	Dr Bob Taylor	Personal communication	Queen Victoria Museum and Art Gallery
Queen Victoria Museum and Art Gallery, Entomology	Lisa Gershwin	Unable to access relevant records due to refurbishment http//www.ozcam.org/cgi-bin/emu-dataportal.cgi//transform/126136664727847 or http//www.biomaps.net.au/biomaps2/	Queen Victoria Museum and Art Gallery
Oxford University Museum Entomology	entomology@oum.ox.ac.uk	No response	Oxford University
Global Biodiversity Information Facility	-	No results for *Myrmecia* http//www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=578252	Various
Natural History Museum	Suzanne Ryder – Collections manager, Department of Entomology Suzanne Ryder <S.Ryder@nhm.ac.uk>	Collection records incomplete (see http//www.nhm.ac.uk/research-curation/collections/departmental-collections/entomology-collections/search/	Natural History Museum, London

Table 4-1 Location information was resourced from the above institutions. Relevant contact and collection details for each institution are shown, including how the records were accessed.

4.3 Results

4.3.1 Known occurrences

A total of 446 usable *M. pilosula* records were assembled (Figure 4-1). This was deemed to be a sufficient data set to have confidence that these were a satisfactory cross section of occurrences for this species complex. A summary of the location data is presented in Table 4-2.

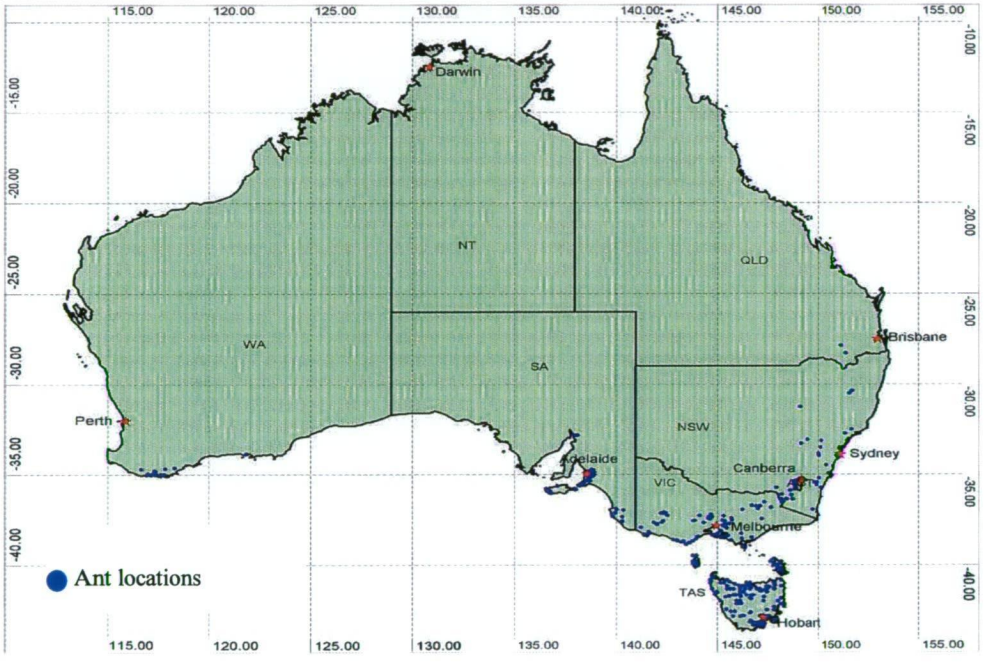


Figure 4-1 Map showing locations where *M. pilosula* have been collected throughout Australia.

	Max	Min
Latitude	27.88S	43.33S
Longitude	151.80E	116.70E
Elevation	1626.10m	0.00m

Table 4-2 Extreme ranges of the 446 known locations of *M. pilosula* complex (in decimal degrees and metres above sea level)

4.3.2 Climate

Thirty-five climate parameters for each location were generated by BIOCLIM. Climate values for each parameter at each location were subjected to a principal component analysis. The first six principle components displayed eigenvalues greater than 1. Even so, four principal components account for 87.38% of the total variance. Components 1 and 2 explain two-thirds of total variance (67.77%) (Table 4-3), although the results of a scree plot suggest PCA 1 has a relatively higher loading than PCA 2 and can therefore be used independently to describe the variation in locations (Figure 4-2).

PCA axis	%	Cumulative %
1	44.92	44.92
2	22.85	67.77
3	12.98	80.75
4	6.62	87.38

Table 4-3 Principle component analysis showing the % contribution of each axis.

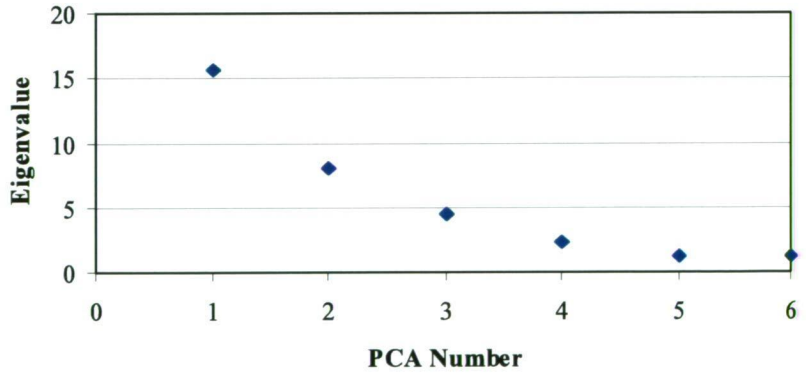


Figure 4-2 Scree plot showing large break in the eigenvalues after 2 axes.

Climate parameters and corresponding loadings are presented in Table 4-4. For the purpose of this study parameters with a loading of greater than 0.2 have been selected as strong loadings. Using these criteria 22 parameters out of 35 could be used to explain most of the variation in climate parameters between locations (Table 4-4).

PCA 1 shows that the parameters with strong, positive loadings are associated with warmer temperatures and parameters with strong, negative, loadings are associated with dry conditions. PCA 2 shows that minimum temperature of coldest period, mean temperature of driest quarter, precipitation seasonality and radiation of driest quarter, all have positive loadings but temperature range (associated with continentality) at each of the locations has a negative loading (Table 4-4); these describe some of the attributes of a Mediterranean climate, typical of large parts of southern Australia. Some parameters identified as being of relatively small influence include isothermality, and mean temperatures of wettest and coldest quarters, in addition to parameters related to radiation and precipitation (Table 4-4).

Output from a scatter plot graphing the 22 parameters with the highest loadings from PCA 1 against PCA 2 shows that there is a strong negative correlation between dry, cold and seasonal effects and a strong positive correlation between warm temperature and radiation parameters (associated with high rainfall), in locations where *M. pilosula* are found. Some of the moisture parameters loaded highly but did not correlate with other parameters (Figure 4-3).

Parameter	PCA 1	PCA 2
Annual mean temperature	0.20576	0.09122
Mean diurnal range(mean(period max-min))	0.12025	-0.21787
Isothermality 2/7*	0.01407	0.10532
Temperature seasonality (C of V)	0.08769	-0.26327
Max temperature of warmest period	0.22134	-0.06208
Min temperature of coldest period	0.10766	0.26181
Temperature annual range (5-6)	0.10907	-0.24898
Mean temperature of wettest quarter*	0.14431	-0.12665
Mean temperature of driest quarter	0.09236	0.2718
Mean temperature of warmest quarter	0.23069	-0.01197
Mean temperature of coldest quarter*	0.15037	0.18899
Annual precipitation	-0.21278	-0.01116
Precipitation of wettest period*	-0.1659	0.08666
Precipitation of driest period*	-0.19412	-0.15772
Precipitation seasonality(C of V)	0.09992	0.22431
Precipitation of wettest quarter*	-0.17237	0.06788
Precipitation of driest quarter	-0.21897	-0.13666
Precipitation of warmest quarter	-0.18632	-0.20012
Precipitation of coldest quarter*	-0.17026	0.11701
Annual mean Radiation*	0.19293	-0.1014
Highest period radiation*	0.16147	0.08882
Lowest period radiation*	0.19785	-0.12377
Radiation seasonality (C of V) *	-0.1669	0.19773
Radiation of wettest quarter	0.05788	-0.30475
Radiation of driest quarter	0.0147	0.25165
Radiation of warmest quarter*	0.16382	-0.03565
Radiation of coldest quarter*	0.19044	-0.11226
Annual mean moisture index	-0.24195	0.01592
Highest period moisture index	-0.0848	0.21385
Lowest period moisture index	-0.23248	-0.09932
Moisture index seasonality (C of V)	0.22446	0.13269
Mean moisture index of high qtr. MI	-0.08882	0.22464
Mean moisture index of low qtr. MI	-0.23566	-0.10375
Mean moisture index of warm qtr. MI	-0.23339	-0.10889
Mean moisture index of cold qtr. MI	-0.10395	0.23365

Table 4-4 Eigenvector values generated by bioclim describing variation between sites. Out of 35 parameters, 22 displayed loadings > (+/-) 0.2% (highlighted). The remaining parameters (*) are considered to be of comparatively small influence.

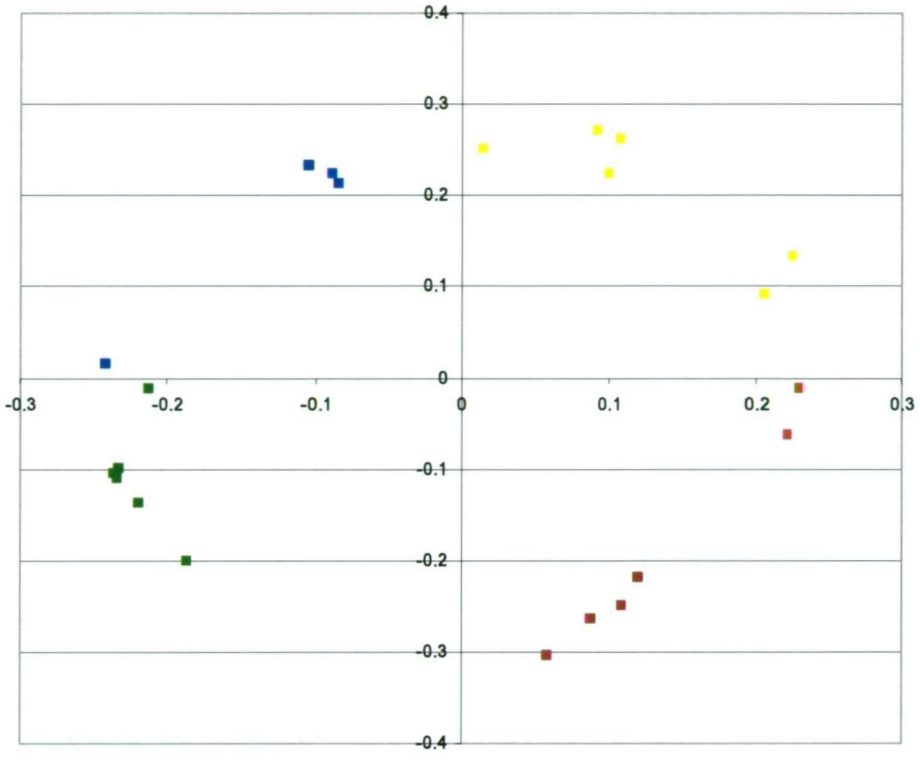


Figure 4-3 Scatter plot showing relationship between Mean moisture index of high qtr. MI; Annual mean moisture index; Highest period moisture index; Mean moisture index of cold qtr. Mean temperature of driest qtr.; minimum temperature coldest period; Annual mean temperature; Precipitation seasonality; Radiation of driest qtr.; Moisture index seasonality MI. Max temperature of warmest period; Mean temperature of warmest qtr.; Mean diurnal range; Temperature annual range; Temperature seasonality; Radiation of wettest qtr. Annual precipitation; Mean moisture index of warm qtr. MI; Lowest period moisture index; Mean moisture index of low qtr. MI; Precipitation of driest qtr.; Precipitation of warmest qtr.

The correlation matrix between the most influential parameters (n=10) in PCA 1 (Table 4-5) demonstrates that precipitation during dry periods and some moisture indices are highly correlated with each other. Although mean temperature of warmest quarter and max temperature of warmest period are also highly correlated (r=0.9275), max temperature of warmest period is not highly correlated with any other parameter. Mean temperature of warmest quarter and annual mean temperature have a strong positive relationship (r=0.918) (Table 4-5). Annual mean moisture index and annual precipitation do not share a correlation co-efficient >0.9 with any

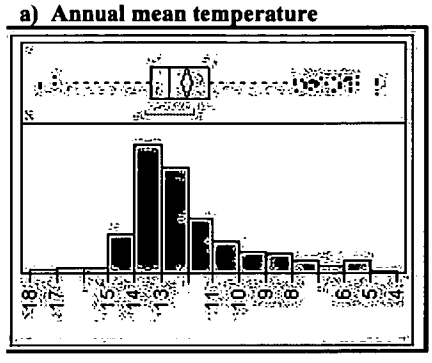
other parameter (Table 4-5)

Out of the 10 highly influential parameters, 6 display stepped frequency distributions that indicate the possibility of threshold-type responses to certain climatic variables. Ants were most commonly collected from locations with an annual mean temperature of less than 15.2°C (97.5%), with 50% of ants being collected from locations with an annual mean temperature range of between 11.2°C and 13.4°C (Figure 4-4a). Ninety percent of ants were collected in locations where the mean temperature of warmest quarter is <19.1°C (Figure 4-4b). Ant occurrence decreases dramatically in locations with annual precipitation of <592mm, with <2.5% of ants being collected outside of this minimum, 87.5% of ants were collected from locations with annual precipitation between 592mm and 1470.9mm (Figure 4-4c). Ants were only present in locations where the precipitation of driest quarter was >58mm (max 473mm; mean=154.65mm) (Figure 4-4d). Three quarters (75%) of occurrences were from locations with an annual mean moisture index of >0.66 (mean=0.74; Max=1.0; Min=0.28) (Figure 4-4e). The frequency distribution of mean moisture index of warmest quarter (MI) had a slight binomial distribution (mean=0.387; Min=0.08; Max=1.0), with 75% of ant locations with a moisture index of <0.51 (Figure 4-4f).

	1. Annual mean temperature	5. Max temperature of warmest period	10. Mean temperature of warmest qtr	12. Annual precipitation	17. Precipitation of driest qtr	28. Annual mean moisture index	30. Lowest period moisture Index	31. Moisture Index seasonality (C of V)	33. Mean moisture index of low qtr. MI	34. Mean moisture index of warm qtr. MI
1. Annual mean temperature	1									
5. Max temperature of warmest period	0.7328	1								
10. Mean temperature of warmest quarter	0.918	0.9275	1							
12. Annual precipitation	-0.6483	-0.6003	-0.6762	1						
17. Precipitation of driest quarter	-0.7466	-0.621	-0.7039	0.8535	1					
28. Annual mean moisture Index	-0.7904	-0.804	-0.8647	0.8281	0.8269	1				
30. Lowest period moisture Index	-0.7847	-0.7285	-0.8045	0.8498	0.9248	0.8594	1			
31. Moisture index seasonality (C of V)	0.7599	0.7217	0.7708	-0.7539	-0.9079	-0.8621	-0.9383	1		
33. Mean moisture index of low qtr. MI	-0.7929	-0.7543	-0.8161	0.8298	0.9361	0.8841	0.9914	-0.967	1	
34. Mean moisture index of warm qtr. MI	-0.7931	-0.7449	-0.8109	0.8086	0.9215	0.8865	0.9804	-0.9754	0.9953	1

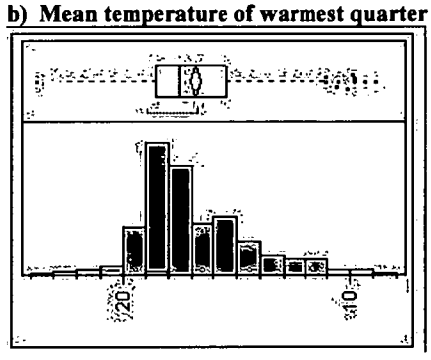
Table 4-5 Correlation matrix between the 10 parameters with the highest loadings, as identified in PCA 1 (Table 4-3). The correlation co-efficients highlighted in light grey are those greater than p=0.9.

a) Annual mean temperature



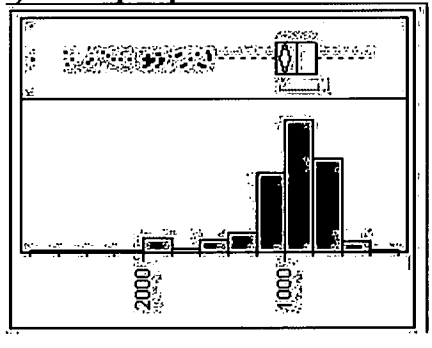
100.0%	maximum	17.5
99.5%		17.38
97.5%		15.20
90.0%		14.10
75.0%	quartile	13.40
50.0%	median	12.70
25.0%	quartile	11.20
10.0%		8.47
2.5%		5.70
0.5%		4.86
0.0%	minimum	4.70
mean		12.03
Std Dev		2.28
Std Err mean		0.10
N		446

b) Mean temperature of warmest quarter



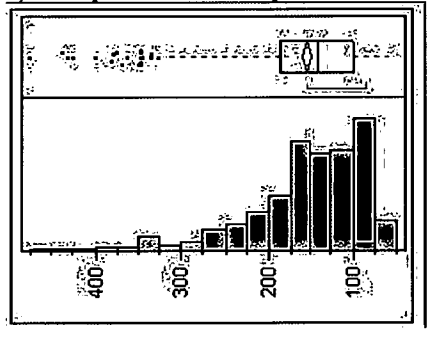
100.0%	maximum	23.60
99.5%		22.80
97.5%		20.66
90.0%		19.10
75.0%	quartile	18.50
50.0%	median	17.50
25.0%	quartile	15.47
10.0%		13.27
2.5%		10.93
0.5%		9.32
0.0%	minimum	8.80
mean		16.83
Std Dev		2.448
Std Err mean		0.11
N		446

c) Annual precipitation



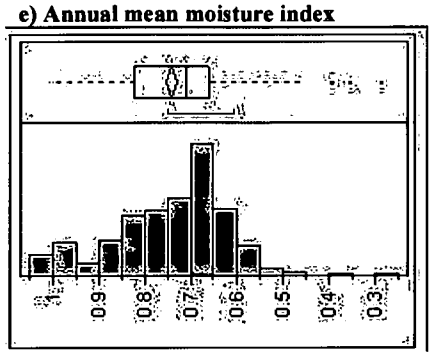
100.0%	maximum	2765.00
99.5%		2528.60
97.5%		2053.80
90.0%		1470.90
75.0%	quartile	1072.50
50.0%	median	917.50
25.0%	quartile	780.50
10.0%		674.20
2.5%		592.00
0.5%		516.30
0.0%	minimum	391.00
mean		1002.39
Std Dev		365.20
Std Err mean		17.29
N		446

d) Precipitation of driest quarter



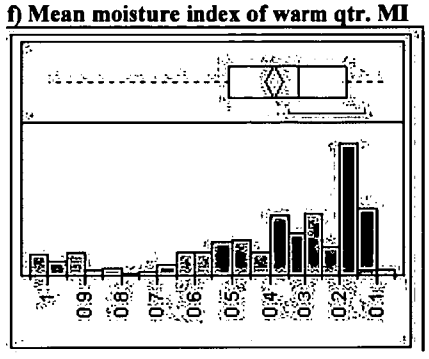
100.0%	maximum	473.00
99.5%		432.36
97.5%		344.83
90.0%		250.60
75.0%	quartile	187.25
50.0%	median	140.00
25.0%	quartile	100.00
10.0%		84.00
2.5%		68.00
0.5%		58.47
0.0%	minimum	58.00
mean		154.64
Std Dev		72.51
Std Err mean		3.43
N		446

e) Annual mean moisture index



100.0%	maximum	1.00
99.5%		1.00
97.5%		1.00
90.0%		0.96
75.0%	quartile	0.82
50.0%	median	0.71
25.0%	quartile	0.66
10.0%		0.61
2.5%		0.54
0.5%		0.35
0.0%	minimum	0.28
mean		0.73
Std Dev		0.12
Std Err mean		0.0059
N		446

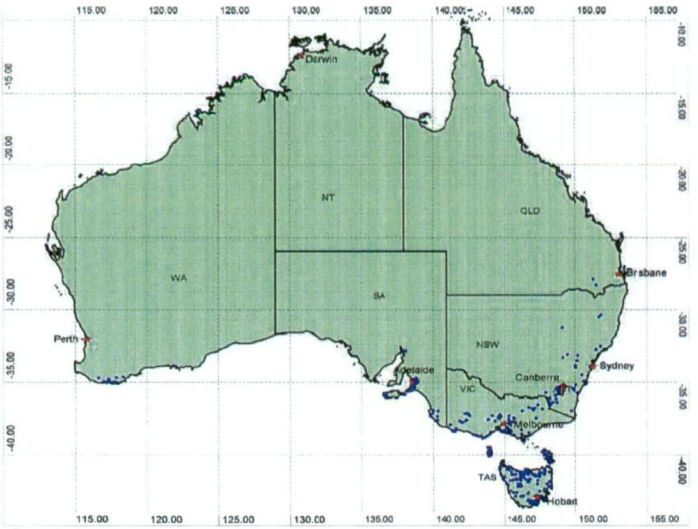
f) Mean moisture index of warm qtr. MI



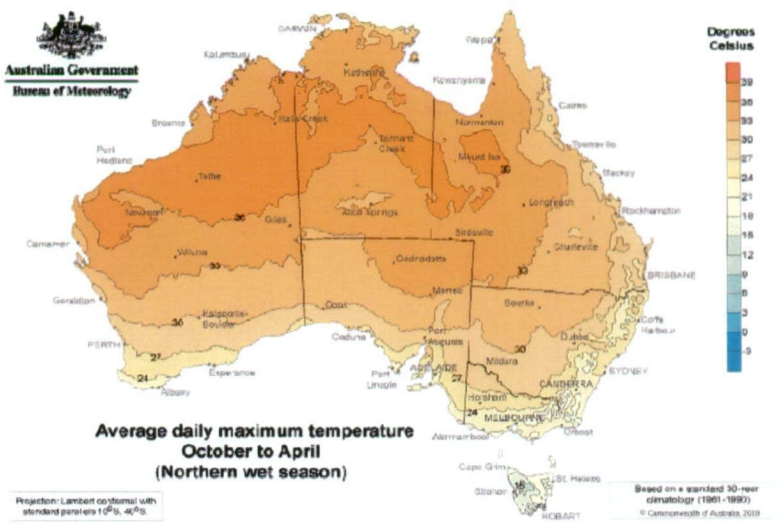
100.0%	maximum	1.00
99.5%		1.00
97.5%		1.00
90.0%		0.89
75.0%	quartile	0.51
50.0%	median	0.32
25.0%	quartile	0.18
10.0%		0.14
2.5%		0.11
0.5%		0.09
0.0%	minimum	0.08
mean		0.38
Std Dev		0.25
Std Err mean		0.01
N		446

Figure 4-4 Frequency histograms and associated descriptive data, indicating possible climate thresholds for 6 of the high loading climate parameters a) Annual mean temperature; b) Mean temperature of warmest quarter; c) Annual precipitation; d) Precipitation of driest quarter; e) Annual mean moisture index and f) Mean moisture index of warm qtr.

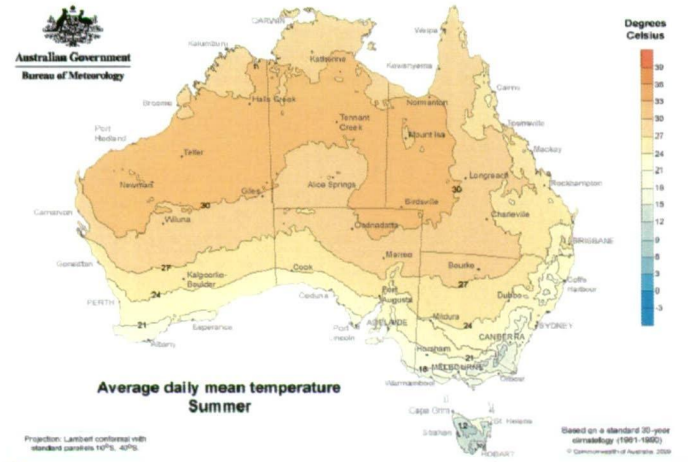
Figure 4-5 visually demonstrates the relationship between high temperatures and *M. pilosula* location. A comparison of Figure 4-5 a, b and c shows that most *M. pilosula* have been collected from areas where the maximum temperature of the warmest period is less than 24°C and maximum temperature of the warmest quarter is less than 21°C. These temperature thresholds neatly encompass the known range of *M. pilosula.* Figure 4-5d shows average annual rainfall values across Australia which also loaded highly. These figures give an easy visual interpretation of some of the results from this chapter.



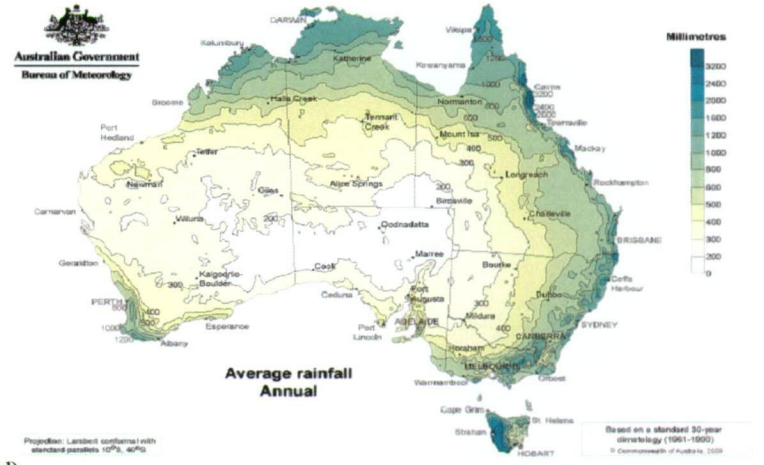
a)



b)



c)



d)

Figure 4-5 Maps of Australia showing a) *M. pilosula* nest locations b) average daily maximum temperature of the warmest period c) Average daily mean temperature of the warmest quarter d) Average rainfall annual (produced by Dr Jon Marsden-Smedley).

4.3.3 Predicted current distribution

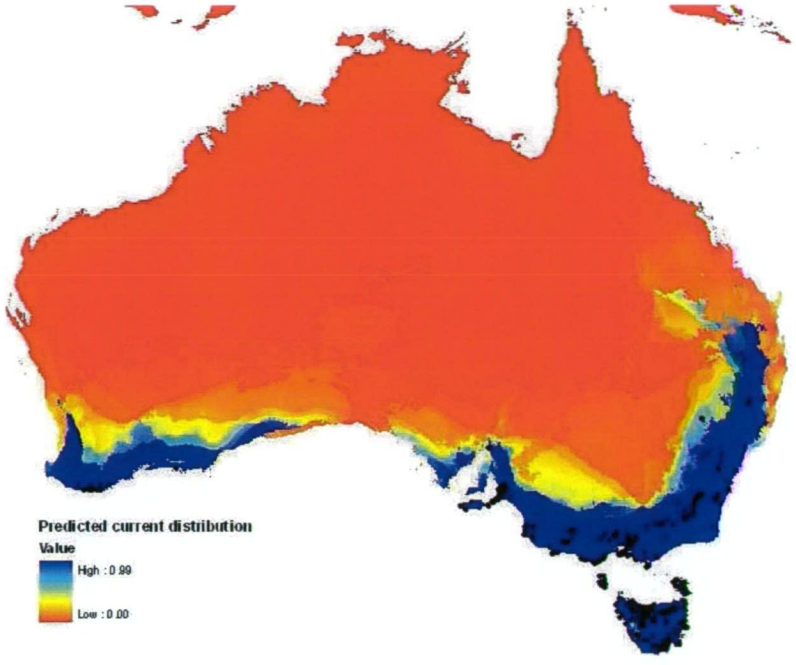


Figure 4-6: Map of current predicted distribution of *M. pilosula*. High values predict a greater probability of occurrence whereas low values predict a small probability. Black symbols are records of *M. pilosula* occurrences

The map of predicted current distribution (Figure 4-6) expands on the range of existing records. A high probability of occurrence exist north and east of Perth, and encompasses a band inland of the coast of southern Western Australia across to South Australia. There is also a high probability of occurrences throughout most of Victoria, eastern New South Wales and all of Tasmania. Probability of occurrences is low throughout most of South Australia except in the extreme south east and south west and most of Queensland except for a section in the south-eastern corner.

4.4 Discussion

The results show that *M. pilosula* occurs in warm, moist environments indicative of southern Australia. The predicted range reflects and extends the current known range and the bioclimatic profile generated by BIOCLIM. Histograms give a more complete picture of how these conditions may affect the distribution of *M. pilosula.* Rather than linear responses, these show that in some cases there are distinct thresholds associated with some climate conditions beyond which far fewer ants have been collected.

Radiation of wettest quarter and mean diurnal range are highly correlated with warm temperature conditions and therefore it could be surmised that these parameters would make useful substitutes as predictors of distribution. Even so, it would be more likely that the datum for temperature would be more readily available for analysis than radiation parameters. Therefore warm temperature conditions have been identified as the most useful surrogate of the climatic envelope in which *M. pilosula* is distributed.

It appears that high temperatures in warmer months may set the upper limit of *M. pilosula* distribution. A similar effect on distribution has been reported in the Argentine ant (*Linepithema humile*) whereby maximum temperature thresholds may constrain their distribution in Australia (Jumbam *et al.* 2008; Walters and Mackay 2004) and South Africa (Jumbam, Jackson et al. 2008). Although some other climatic parameters were also consistent with *M. pilosula* distribution, investigations showed that high temperatures during warmer months are the best predictor of the range of *M. pilosula.*

While the PCA indicated that *M. pilosula* occurrence was influenced by rainfall the associated parameters are not indicative, in isolation, of *M. pilosula* distribution. For example, the histogram for annual rainfall (a parameter that loaded highly in the PCA), shows that approximately 97% of all ant records were located in areas which received between 391mm and just over 2000mm of rainfall. This rainfall range encompasses almost half of Australia; including many areas where *M. pilosula* has never been collected (Figure 4-5d). Annual rainfall was not highly correlated with any other high loading climate factor that could be used to explain the effect of

annual rainfall on *M. pilosula* distributions. Therefore the use of this factor to describe the climatic envelope of *M. pilosula* is limited.

The most useful parameter identified in this study for describing the bioclimatic envelope of *M. pilosula* is maximum temperature during the warmest part of the year. Not only did it load highly, the meaning of this parameter is quite clear, unlike some such as insolation, which is difficult to interpret and relate to on a large scale. Also, the data associated with temperature parameters in Australia are readily available from the Bureau of Meteorology website which allows easy access for visually describing the potential range.

This study has advanced the ability to describe the environmental features within the range of *M. pilosula*. Previous descriptions have been vague and untested. The predicted distribution modelled and thresholds described in this study could be tested by targeting sampling along the predicted limits and climate thresholds. The climatic envelope described here could be used to guide more detailed studies on other factors that may limit *M. pilosula* distribution e.g. soil (Way *et al.* 1997) and vegetation type (Lindenmayer *et al.* 1991). This could be achieved by collecting presence and absence data along with soil and vegetation types, along transects of latitudinal gradient from outside of the predicted distribution to within.

The collection records used in this study were believed to give a reasonable representation of the *M. pilosula* distribution. But it is clear from the map of predicted distribution that there are large areas where it is likely *M. pilosula* occurs but has not been collected. This deficit in certain parts of Australia may be indicative of the vetting process used to collate the records for this study. It is possible that some records that were discarded due to a lack of information are actually specimens of *M. pilosula* complex but because of labelling or identification inaccuracies they could not be utilised. Or, the deficit could also be a product of collection bias towards similar localities which has resulted in those locations being over-represented. This bias could have occurred due to the directed collection at the most accessible localities, or where there are known occurrences. For example the specimen set held by the Jack Jumper Allergy Program were collected by targeting particular habitats across southern Australia where it was believed there would be a

high likelihood that *M. pilosula* would be present.

BIOCLIM and MaxEnt were both employed for modelling purposes. For future studies with presence only data, MaxEnt could be used for both bioclimatic and predictive modelling using the climatic parameters from Worldclim. The benefits of this are a simplification of data manipulation, and testing of fewer climatic parameters (limited to 19 in Worldclim, as opposed to 35 in BIOCLIM). Although this method may restrict the identification of key climate parameters within the generated bioclimatic profile, it is likely that those not accounted for by Worldclim will be highly correlated with those that are, rendering them largely redundant. The detailed benefits and limitations of both modelling packages are described succinctly by Steiner *et al* (2008).

Chapter 5 The activity of multiple *M. pilosula* colonies and local resource availability

Abstract

This study determined the general pattern of above ground daily activity levels at multiple nests of *M. pilosula* at a site near Hobart, Tasmania. Levels of activity were observed throughout the day and demonstrated a bimodal pattern, with a higher number of ants active in the morning than the afternoon. Nest surface size was also measured (as an indicator of colony size) and although nest size showed a slight relationship, it was not a good indictor of nest surface activity. This study identified eight behaviours displayed by ants on the nest surface with the conclusion that there were generally more ants observed on the nest surface throughout the day than there were actually exiting the nest (foragers). Arboreal sampling showed that there is a relationship between plant types and *M pilosula* presence in the spring, but a relative lack of *M. pilosula* on vegetation in the autumn reduced the ability to make inferences about relationships at this time of the year. This may be due to a reduction in food requirements within the colony between spring and autumn. The activity of *M. pilosula* colonies in their native habitat has not been previously studied. This record of daily activity cycles is valuable information to increase public awareness of the times of day that there would be high risk of an interaction with *M. pilosula* and a consequent sting event; in this case, in the mornings at the nest surface, and during the middle of the day away from the nests. Two limitations of the methods in this study have been identified: 1) The variation between levels of activity between each nest makes it difficult to quantify actual activity levels over time; 2) As a result of large distances between nests and the unpredictable amount of time it took to make observations it is likely that some activity at the nest surfaces was not witnessed due to the infrequency of observations. A more consistent number of observations at each nest may have enabled greater over all clarity of nest activity but would have been impractical to employ. This study was useful as a basis for gaining general knowledge about the daily activity of *M. pilosula* but it highlighted the need to gather further information at a greater resolution to generate more conclusive outcomes.

5.1 Introduction

Many aspects of ant behaviour and activity have been well described in relation to invasive species and competitive dynamics (Holway *et al.* 2002a; Human and Gordon 1999). From an ecological perspective, the impact of ants on soil properties and biota (Boulton and Amberman 2006; Cowan 1985; Folgarait 1998) and their use as restoration or environmental indicators has also been widely studied (Andersen 2004; Hoffmann and Andersen 2003; Majer 1983). Information on a specific ant species is more limited unless a researcher has taken a particular interest that ant e.g. *Camponotus* spp. Creighton (1951; 1952; 1953; 1965; 1969). Sometimes this interest is limited to taxonomic or molecular studies of the ant (McArthur 2003; 2009; McArthur and Adams 1996a; b; McArthur *et al.* 1997; McArthur and Shattuck 2001).

Although *M. pilosula* is one of the most easily recognised ants in Southern Australia, there has been very little research into its daily activities and behaviour. Studies such as Haskins and Haskins (1950) and Gray (1971a) provide interesting narrative on the behaviours of many *Myrmecia* species including *M. pilosula.* Other researchers have observed *M. pilosula* in laboratory conditions (Freeland 1958; Haskins and Haskins 1980). These studies provide information on various traits such as foraging behaviour (Gray 1971a; Morrison 1983), pupal eclosion (Haskins and Haskins 1950), and feeding behaviour (Freeland 1958). None of these describe the daily behaviours of *M. pilosula* or attempt to explain what environmental conditions influence its levels of activity.

Examining the behaviour of ants can offer valuable scientific knowledge of the environment around us. The study of the behaviour of *M. pilosula* has a practical application for humans that share the same territory as the ant but wish to avoid it. *Myrmecia pilosula* is a native ant endemic to southern Australia, therefore options for chemical control of the ant are limited if the natural ecosystem is to be preserved. Most private land owners with nests in their yard apply boiling water directly on to nests, or petrol to deter or exterminate them. These solutions are not practical for long term management of ant-human interactions, nor suitable to maintain a healthy environment. A greater understanding of the behaviour and activity of *M. pilosula* at

the nest surface could reduce interactions between the ant and the human community without relying on individual nest treatments.

For this purpose, this study examines whether there is a pattern of nest activity that can be predicted over the course of a day. The method of prediction will have to be applied by the public to be useful. Therefore easily identifiable visual cues, which can be easily examined in terms of ant activity should be used, such as; the size of the nest surface (as an indicator of colony size), or time of day, could be functional and useful predictive tools.

The above ground behaviours most frequently displayed by *M. pilosula* at a particular time of the day will determine their level of exposure to the human community during that time. For example if human outdoor activity occurs at the same time of the day as ant activity outside the nest (e.g. foraging ants) there is greater risk of exposure. Or, if activity at the nest surface is especially high during a particular time of the day, the risk of a sting event would be greatest if a person actually came into contact with a nest during this period. Ants perform different roles within the colony, which can be classified by the types of behaviour an ant displays (Buckingham 1911; Holldobler and Wilson 1990). Some recognised behaviours include foraging (leaving the nest to search for food) and nest maintenance (the movement of nest material). These behaviours should be easily determined in this conspicuous ant and would assist in understanding where ant activity is likely to be focussed at different times of the day i.e. on the nest surface or away from the nest.

Habitat type may also be an indicator of relative activity levels of *M. pilosula* which could be determined by examining the relationship between vegetation type and arthropod prey (Hill 2003). For example, foraging in two species of *Myrmecia* has been observed in trees of *Eucalyptus largiflorens* where they searched for nectar and prey (Gray 1971b). *M. pilosula* has also been observed collecting 'manna' excretions from *Eucalyptus viminalis* (Muir 1974) which only the adults utilise.

This chapter aims to discover and characterise common behaviours displayed by individuals in colonies of *M. pilosula*, in addition to describing the cycle of ant activity during the day. This will specifically address whether level of activity is

associated with time of day or size of nest surface. Arboreal host plants and associated potential prey items are also investigated for their influence on ant activity.

5.2 Methods

A total of 22 nests were located at Hobart College Campus grounds on Mt Nelson, Hobart, Tasmania (Figure 3-3). Each nest was identified using the methods described in Chapter 3. The nest surface was photographed and the surface area was measured. Behavioural observations on ants were taken at each nest, at regular intervals, during one day. Ambient temperature and relative humidity were recorded throughout the day to determine if weather conditions were limiting.

5.2.1 Description of nest surface

For this study the nest surface was defined as the identifiable above ground features of the nest. The nest surface of *M. pilosula* usually consists of a mound decorated with small, sorted pebbles, seeds and twigs of consistent sizes and shapes. The nest surface usually has a distinctive perimeter boundary. For the purposes of this study the nest surface is the area within this perimeter (Figure 5-1) and is easily distinguished from most other ant nests. For example, *Polyrhachis hexacantha* nests in the same habitats as *M. pilosula* and makes a low mound nest of similar size, but it lacks gravel decoration and consists largely of fine plant material.

Figure 5-1 Photograph of a nest used in this study showing perimeter and decoration of nest surface.

Nest surface area was measured by using the following formula:

Nest surface area = length x width

The above formula was used to calculate all nest surface areas, even though they were often of inconsistent shapes. The formula was considered most appropriate because of its ease of application, and because most nests consist of a range of shapes from rectangular to ovoid with only a slightly raised mound. It was considered that, for the purpose of this study, the margin of error was negligible and that a reliable index of relative nest area was obtained.

The length of the nest was measured using callipers, which were extended across the largest diameter of each nest. The distance between each arm of the calliper was then measured using a standard measuring tape. The width of the nest surface was measured using the same technique except the callipers were extended across the middle of the nest surface, and at right angles to the length measurement (Figure 5-2).

Figure 5-2 Technique used to measure nest length and width. The orange flagging tape is resting on the top of the nest.

5.2.2 Behavioural observations

Behaviours were identified from direct observation and allocated a number between 1 and 8 (Table 5-1). Nest observations were made on the 3rd November 2009. Observations were recorded, between 0710 and 1845 (Eastern Australian Summer Time), by walking between nests in random order, tallying the number of *M. pilosula* on the nest surface and recording the ranges of behaviours they were displaying.

Nests were only observed for as long as it took to count the ants on the nest, record their behaviour and collect any individual carrying prey.

Number	Behaviour	Behaviour description
0	No ants present	No ants seen
1	Sentry	Ants remaining at the entrance hole of the nest
2	Wandering	Ants on the surface of the nest but without any particular purpose
3	Nest maintenance	Ants observed carrying nest surface material on nest surface
4	Carrying food	Ants returning to the nest with prey
5	Carrying JJA	Ants carrying another individual from the nest
6	Entering nest	Ants entering nest from greater than 0.5m away without prey
7	Exiting nest	Ants leaving nest surface (assumed to be a forager)
8	Within 0.5m from nest	Ants that were observed within 0.5 of the nest surface

Table 5-1 Numbers and description assigned to each behaviour

5.2.3 Climate conditions

Ambient temperature and relative humidity readings were measured using a Kestrel ®, hand-held weather station. Recordings were taken every half hour, or as close to every half hour as possible, resulting in 22 recordings for the day.

5.2.4 Arboreal food resources

To determine resource availability for foraging ants, vegetation beating took place within 20m of nests (located as described in Chapter 3) on the 23rd November 2009 (spring sample) and the 17th March 2010 (autumn sample). Both days were mild and sunny, such that weather conditions were not considered a limiting factor for ant activity. Only shrubs or trees above 0.5m in height having branches within arm's reach were sampled. Between two and five samples from each plant type were collected on each occasion. A sample consisted of 10 beats per plant type, whereby leafy branches of the plant were beaten once with a heavy stick. If it was not possible to collect one entire sample from one plant then the sampling was completed on an individual of the same species within close proximity. A sample of each plant was collected to confirm its identification. The arboreal invertebrates from each

plant were concentrated on a white plastic tray as they were dislodged from the foliage during the beating process. The invertebrates were then transferred to a sample container and preserved in 70% ethanol for later identification in the laboratory (Appendix 1).

5.2.5 Data analysis

The mean number of ants on each nest per observation period and the frequency of various behaviours were calculated. This data is presented in graphical form. Correlations between nest size and ant activity, weather conditions, activity and behaviour type are explored. Multivariate relationships between the presence of *M. pilosula*, plant type and potential prey on each plant type are examined using the computer program PC-ORD (McCune and Mefford 1999).

5.3 Results

5.3.1 Nest observations

The average temperature was 15.8°C and the average relative humidity was 79%. These conditions were not considered limiting to activity or behaviour of the ants at the nests. A total of 22 nests were observed throughout the day. Each nest was observed between 5 and 7 times during the day, with 9 nests observed every half hour. No observations were made between 1030 and 1100 or between 1630 and 1730. The total number of *M. pilosula* observed was 395, with a mean of 2.9 ants per nest observation (S.E. = 0.36; n=135), displaying a mean of 1.34 behaviours (S.E. 0.16; n=181). The nest with the highest number of ants observed was nest 21 (n=87), the nest with the lowest number was nest 16 (n=0) (Figure 5-3). The total number of behaviours observed was 8 (this includes 'no ant' observations). Behaviour 2 (wandering) was observed most frequently (n=42), in addition to behaviour 0 (no ants at nest surface) (n=42) (Table 5-2). Behaviour 5 (carrying another ant) was observed the least frequently (n=1).

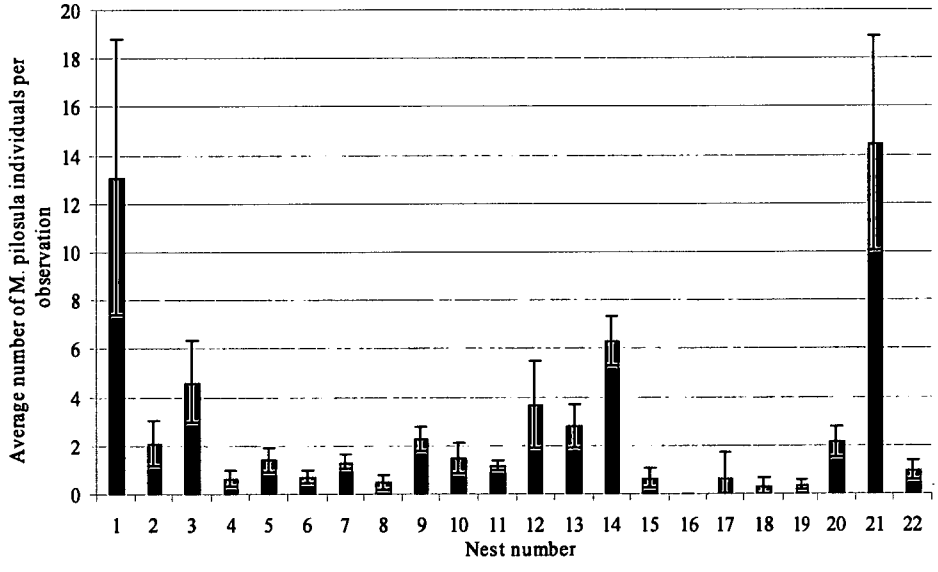


Figure 5-3 Mean number of ants on each nest surface per observation during 1 day.

Task	Frequency observed	Histogram

7	19	
6	8	
5	1	
4	3	
3	32	
2	42	
1	34	
0	42	
	n=181	

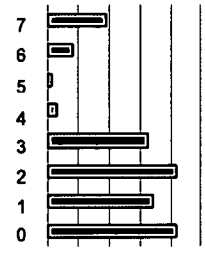


Table 5-2 Task, frequency observed and histogram showing how often each task was observed over 1 day. 0=No ants present, 1=Sentry, 2=Aimless wandering, 3=Nest maintenance, 4=Carrying food, 5=Carrying another *M. pilosula*

The greatest numbers of ants were observed between 0830 and 0930. The least ants were observed between 1130 and 1430 with less than 5 ants observed each half hour over a three hour period (Figure 5-4). Ant numbers fluctuated throughout the rest of the day (Figure 5-4). The greatest number of ants displayed behaviour 2 (wandering) (n=226) followed by behaviour 3 (nest maintenance) (n=72). The least number of ants displayed behaviour 5 (carrying another ant) (n=1) (Figure 5-5).

Sentry (behaviour 1) and wandering (behaviour 2) were displayed fairly constantly throughout the day with only 4 half hour periods (n=21) when there was no sentry observed (behaviour 1) and only 3 half hour periods (n=21) during which no aimless wanderers (behaviour 2) were observed. Behaviour 2 was at its highest in the morning between 0830 and 1000. Behaviour 3 was not observed before 0900, or after 1830. Ants returning to the nest with prey (behaviour 4), were only observed three times during the day, with no obvious pattern in timing. Behaviour 5 (carrying another ant) was only observed once. Ants started returning to the nest without prey (behaviour 6) after 0800 and continued until 0900, and then inconsistently between 1200 midday and 1830. Behaviour 7 was only observed between 0800 and 1130 and between 1300 and 1600 with one ant leaving the nest after 1600 (Figure 5-6).

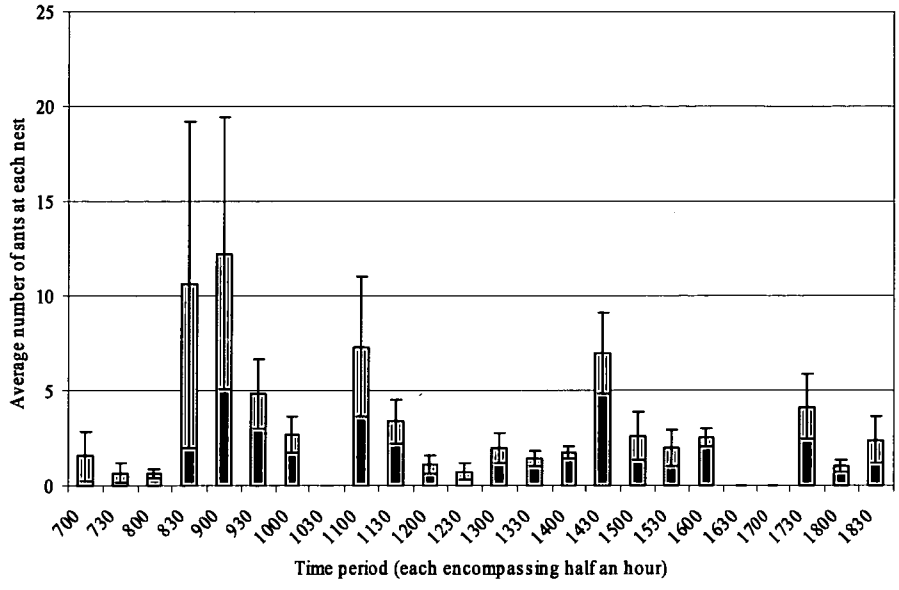


Figure 5-4 Mean number of ants observed at each nest during every half hour period (+/- S.E.).

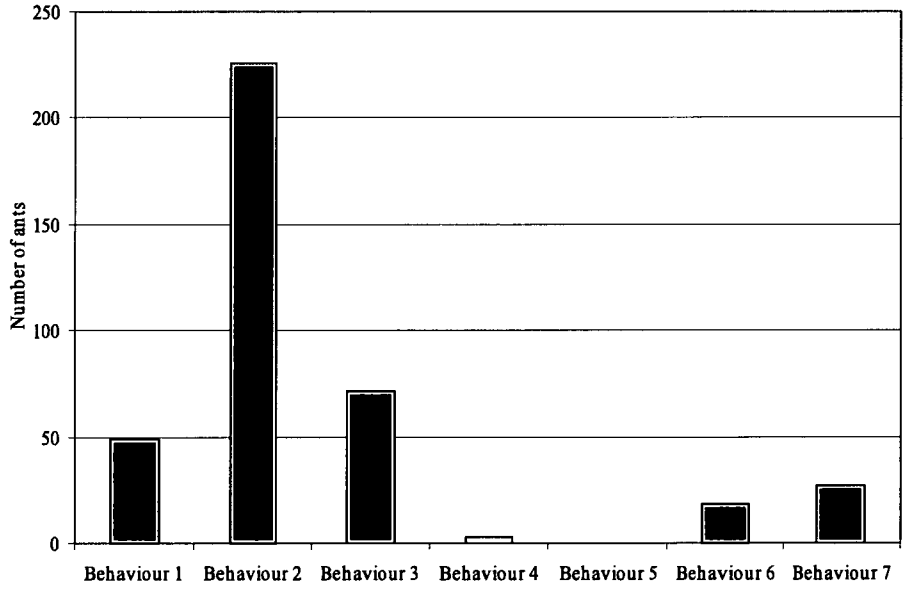


Figure 5-5 Total number of ants displaying behaviours. Behaviour 1=Sentry, Behaviour 2=Aimless wandering, Behaviour 3=Nest maintenance, Behaviour 4=Carrying food, Behaviour 5=Carrying another *M. pilosula*, Behaviour 6= entering nest with no prey, Behaviour 7 = Exiting nest.

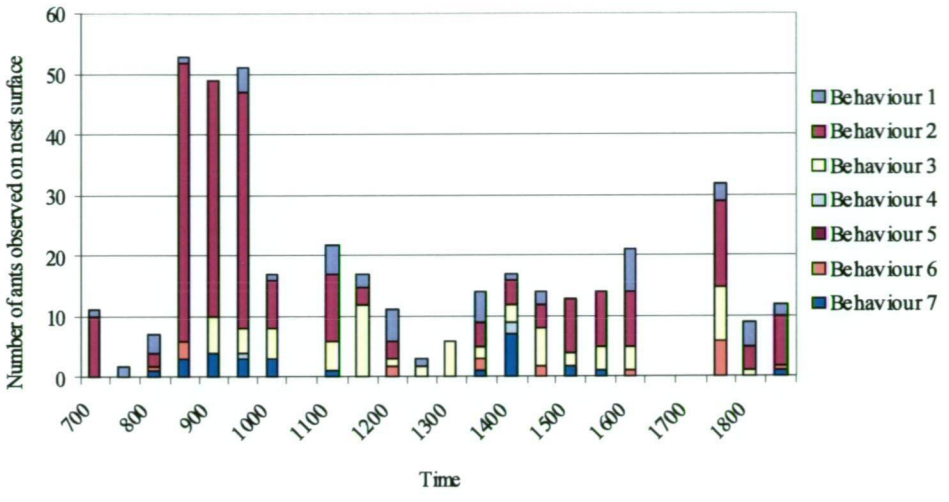


Figure 5-6 Number of ants on nest surface and type of behaviour displayed over 1 day. 0=No ants present, 1=Sentry, 2=Aimless wandering, 3=Nest maintenance, 4=Carrying prey, 5=Carrying another *M. pilosula*; 6=Entering the nest (without prey); 7=Exiting the nest.

There was a positive correlation between ant activity and nest surface area (r= 0.61), but this relationship was strongly driven by a few large outliers (Figure 5-7).

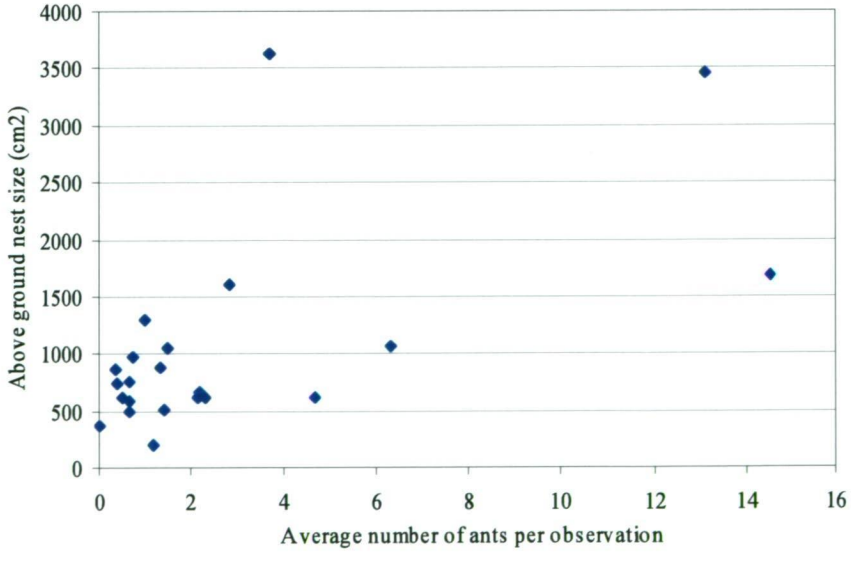


Figure 5-7 Scatter plot showing slight positive correlation between size of nest surface area and ant activity

5.3.2 Arboreal invertebrate sampling

A total of 11 most common woody plant species were sampled with 63 invertebrate taxa collected (Table 5-3). In November 2009, 7 plant species were sampled with 37 invertebrates collected and a mean of 7.1 invertebrates per plant. In March 2010, 12 plant species were sampled with 51 invertebrates collected and a mean of 8 invertebrates per plant.

Plant type	Mean number of invertebrates
Acacia dealbata	12.3
Acacia melanoxylon	4
Acacia stricta	8
Allocasuarina	5
Dodonaea viscosa	6.3
Epacris impressa	8
Eucalyptus ovata	3.5
Eucalyptus pulchella	4.7
Exocarpos cupressiformis	10.75
Exocarpos strictus	6
Leptospermum scoparium	8

Table 5-3 Mean number of invertebrates collected from each plant species.

In spring 2009, *M. pilosula* was collected from the foliage of *Acacia stricta, Acacia melanoxylon, Eucalyptus ovata, Eucalyptus pulchella* and *Leptospermum scoparium.* In autumn, the ant was collected from *Acacia stricta* and *Exocarpos cupressiformis.* Of these plants, the highest mean number of invertebrates was collected on *Exocarpos cupressiformis.* On average, there were significantly more invertebrates on shrubs with *M. pilosula* (mean = 50.5 +/- 2.4) than without (mean = 21.7 +/- 0.99) ($t_{1,28}$=1.656, p=0.054).

Ordination analysis of the spring sample shows that *Thudaca* moth larvae, two types of spiders, (a Gnaphosidae, and a Salticidae), a thrip, and *M. pilosula* is strongly associated with *Acacia stricta, Leptospermum scoparium* and, to a lesser extant, *Eucalyptus pulchella* (Figure 5-8). In autumn 2010, the strongest relationships were between five types of arachnids, and the plant taxa *Leptospermum scoparium, Acacia stricta, Acacia dealbata,* and to a lesser extent, *Epacris impressa* and *Exocarpos cupressiformis* (Figure 5-9). There is no indication that *M. pilosula* is strongly associated with any particular plant or insect collected in the autumn samples.

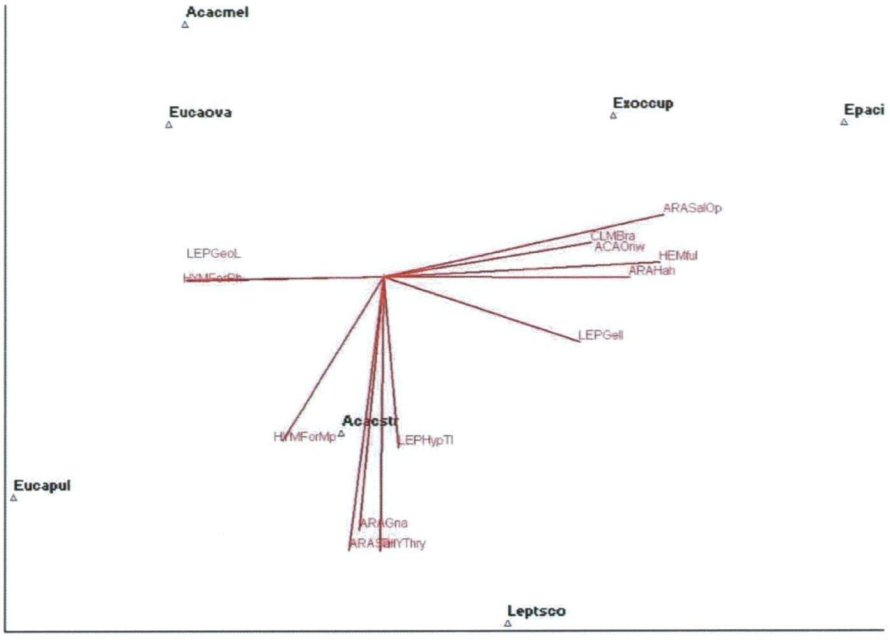


Figure 5-8 Ordination showing strong associations between invertebrates found on *Eucalyptus pulchella, Acacia stricta* and *Leptospermum scoparium* collected in spring 2009. Stress in 3D = 17.3%. See Appendix 1 for invertebrate codes.

Figure 5-9 Ordination showing strong relationship between invertebrates collected on *Leptospermum scoparium, Acacia stricta,* and *Acacia dealbata* in autumn 2010. Stress in 3D = 14.6%. See Appendix 1 for invertebrate codes.

5.4 Discussion

This study found that activity levels differed between nests and was only slightly correlated with nest size but not time of day. In general, the least amount of ant activity was observed during the middle and end of the day. This lack of activity during this period could be a response to heat, radiation or because of a change in task priority over that time period (Cloudsley-Thompson 1989; Curtis 1985; Hoffmann 1998).

Most of the ants observed during the most active period of the day (0830 and 0930) appeared to be engaged in 'wandering'. They walked on the surface of the nest, occasionally pausing for a brief moment and then moving again. Due to the short period of observation at each nest it was difficult to determine whether these ants were subsequently employed to perform a particular task. Considering the individual ant's altruistic nature (Holldobler and Wilson 1990), it could be assumed that this behaviour has a purpose which contributes to the colony well-being. It is possible that their behaviour is part of an assessment to determine overnight damage to nest surface structure, to gauge weather conditions or to engage visual cues to orient themselves with nest surface structure and other environmental features. They may also use the heat absorbed by the nest surface to warm up before beginning foraging activity. However, while the frequency of this behaviour was highest in the early morning, it continued to be the most frequently observed behaviour throughout the day.

The actual number of individuals foraging during any part of the day seems to be low compared to individuals displaying other behaviours on the nest surface. This suggests that the greatest chance of encountering an ant would occur if a person came into contact with the nest, or within close enough range to elicit an aggressive response. In contrast, the chance of encountering a foraging ant away from the nest is relatively small. The results suggest that the time of least risk of an encounter with a foraging ant would be prior to 0800, before the ants have left the nest to forage, or after 1800 when a high proportion of foragers have returned to the nest. The possibility of a person encountering a foraging ant would also partly depend on the

number of nests in the area and the foraging range of *M. pilosula.*

Foliage sampling shows that many of the common shrubs in the vicinity of nests host a wide range of invertebrates suitable as potential prey for the ants. This was especially evident from the spring sampling results which found strong associations between *M. pilosula* and particular species of woody vegetation and invertebrates.

There were fewer *M. pilosula* collected in the arboreal autumn samples compared to spring, even though potential prey items were more abundant. Ant activity is likely to reflect the demand for protein in the nest and so it might be expected that ant activity on foliage will peak at times when seasonal larval numbers are at their highest. This decrease in foraging activity by *M. pilosula* in the colder months could be driven by a seasonal reduction in brood productivity within the nest in colder months (Gray 1974), and therefore a decline in protein requirements.

The ordination analysis shows that most shrubs have a particular profile of invertebrates which is characteristic of the host plant. This is to be expected to some degree, given the importance of plant biochemistry as a regulator of insect herbivory (Gulsen *et al.* 2010; Lindroth 2010). However, the physical architecture of plants arising from the dimensions and arrangement of stems and foliage may play an important part in the determination of invertebrate populations associated with a particular plant (Peeters 2002; Riihimaki *et al.* 2006). Thus, *Exocarpos cupressiformis* offers a fine-grained living environment to invertebrates due to its closely packed, pendant photosynthetic stems, small leaf scales and short internodes. This may explain the large numbers of invertebrates found on this plant per volume of foliage. As a consequence it could be suggested that *M. pilosula* and potential prey could be associated with plant structure rather than a particular plant species.

In the context of potential food resources, this study has not explored potential nectar or other liquid food resources of which *M. pilosula* might take advantage. It is expected that *M. pilosula* are more likely to be utilising these resources during peak flowering season, but may also exploit plants that flower outside of this time. A targeted study of flowering plants across seasons would be useful to determine other plant associations that *M. pilosula* may have.

To enable greater resolution of ant activity over one day, one of the most active nests was chosen for a more detailed examination. The following chapter focuses on the effects of climate conditions on behaviour and surface activity. Prey type returned to the nest is also examined with comparisons to arboreal sampling results from this chapter.

Chapter 6 Effects of weather conditions on colony activity and behaviour

Abstract

The aims of this chapter were to conduct a more detailed investigation into daily activity patterns between two seasons, spring and autumn and the relationships between activity and weather factors at the nest. This study demonstrated that activity at colony level could be predicted throughout the day in both seasons, with a similar pattern of activity displayed at both times of the year, but the nest was less active in the autumn. The profile of observed behaviours throughout the day differed from previous results whereby activity at this nest was at its peak in the morning with most of the ants during this time engaged in 'wandering'. This pattern of activity and the behaviours displayed were not correlated with any of the measured variables, but there does appear to be a relationship between activity and solar radiation which is supported by previous research on *Myrmecia* and other ants. Foraging continued throughout the day in the spring, with the majority of foraging ants leaving the nest in the morning and returning in the evening. In the autumn, nest activity came to a halt during the middle of the day. Other behaviours were displayed continuously throughout the day on the nest surface in spring, but there was an obvious difference in activity levels between spring and autumn. Results indicated that some ants continue foraging after dark. Prey items intercepted at the nest varied, but the ants had a preference for Chrysomelidae Paropsini A larva in the spring. These results are discussed in relation to seasonal brood production and protein requirements within the colony. These results have implications regarding the times of day that the public are more at risk of a sting event in the vicinity of a nest.

6.1 Introduction

Continuous field observations of a single ant colony have not been conducted. Field observations of ant colonies have been conducted but at short time intervals (Hoffmann 1998), which as discussed in the previous study Chapter 5, may not give an accurate description of activity.

Studies have shown that ant activity is controlled by a number of climatic and seasonal conditions (Holldobler and Wilson 1990; Holway *et al.* 2002b). Correlations between activity and climate have been found in many ants including *Iridomyrmex spp.*, *Melophorus aenovirens*, *Messor barbarus* and *Pogonomyrmex occidentalis* (Azcárate 2007; Bucy and Breed 2006; Cloudsley-Thompson 1989; Hoffmann 1998). More specifically, weather conditions have been found to regulate task allocation (Hoffmann 1998) although weather conditions may not be used in all circumstances as an indicator (Azcárate 2007). On the basis of this evidence, it is therefore likely that local weather conditions also affect activity in *M. pilosula.* In particular, foraging behaviour is one aspect of ant activity that will determine the level of human interaction with *M. pilosula.* Therefore it would be beneficial to accrue information on the effect of weather conditions on nest activity and subsequent behaviours.

As described in the previous chapter, the daily activity patterns of *M. pilosula* have not been explored thoroughly. Some behaviours I have identified have unknown purposes or benefits e.g. 'wandering' on the nest surface. As discussed, wandering behaviour may be related to orientation, heat absorption and damage assessment. Other behaviours that were identified in Chapter 5 are common amongst ants and have obvious benefits to colony function e.g. foraging. The intensive observation of an individual nest has the advantage of gaining insights into the purpose of behaviours that are not well understood. This is in addition to gathering intimate details of how different aspects of colony activity will affect the risk of contact with humans.

Whereas Chapter 5 considered overall ant activity at a number of nests, this chapter focuses on the behaviour that individual ants display on the nest surface and how these may contribute to colony function. In addition, I consider how weather

conditions broadly affect the ant's behaviour. Observations are also made on the types of prey returned to the nest over the course of a day, and comparisons are made with results from Chapter 5.

6.2 Methods

6.2.1 Nest observations

Observations were taken at a selected nest on Mt Nelson, Hobart (Figure 6-1). This nest was chosen because of its high level of activity (see Chapter 4). *M. pilosula* activity was observed between 0530 and 0800 on the 24th Nov 2009 and between 0800 and 2000 (Eastern Australian Summer Time) on the 25th Nov 2009 (spring), also on the 24th March 2010 (autumn) between 0700 and 1930. Although observations were taken on separate days in the spring, it was determined that weather conditions were very similar on both days and therefore the difference between days would have very little impact on ant activity (Table 6-1). There were no conditions experienced that would limit ant activity or behaviour during the spring or autumn observations.

Figure 6-1 Target nest showing distinctive nest surface area.

Individual ants on the surface of the nest were counted and scored for their behaviour. A colour marking scheme was applied which coded for their observed behaviour at the time (Table 6-2). The nest surface is defined as previously in Chapter 3 (Figure 6-1). The nest was observed from a safe distance to avoid evoking defensive behaviour and I varied my location around the nest so the ants were not attracted to a particular location, and so that my shadow fell away from the nest at all times.

Climate Conditions		24 Nov	25 Nov
Temperature (C)	Minimum	12.5	16.1
	Maximum	24.9	25.3
Rain (mm)		0	0
Evaporation (mm)		4	9.2
Sun (hrs)		10.9	13
Sunrise		0501	0500
Sunset		1954	1955
Max wind gust (km/hr)	Direction	NW	NNW
	Speed	72	63
	Time	1010	103
at 0900	temperature (C)	16.5	19.3
	RH (%)	53	43
	Cloud (8th)	7	7
	Wind Direction	NNW	NNW
	Speed (km/hr)	31	30
	MSLP (hPa)	1016.6	1015.7
at 1500	temperature (C)	24.4	24.9
	RH (%)	40	35
	Cloud (8th)	4	1
	Wind Direction	NNW	NNW
	Speed (km/hr)	24	22
	MSLP (hPa)	1015.5	1015.8

Table 6-1 Climate conditions on the 24th and 25th November 2009. Reproduced from the Bureau of Meteorology website (www.bom.gov.au). Time of sunset and sunrise calculated using a Geoscience Australia calculator (http//www.ga.gov.au/geodesy/astro/sunrise.jsp)

Code	Behaviour	Behaviour description	Paint colour
0	No ants present	-	-
1	Sentry	Ants remaining at the entrance hole of the nest	-[1]
2	Wandering	Ants on the surface of the nest but without any apparent purpose	Orange
3	Nest maintenance	Ants observed carrying nest material on nest surface	Yellow
4	Carrying food	Ants returning to the nest with prey	-[2]
5	Carrying JJA	Ants carrying another individual from the nest	-[3]
6	Entering nest	Ants entering nest from greater than 0.5m away without prey	-[4]
7	Exiting nest	Ants leaving the nest surface (assumed to be a forager)	Blue
8	Within 0.5m from nest	Ants that were observed within 0.5 of the nest surface	-[5]

Table 6-2 Observed behaviours and corresponding paint colour. [1]The entry hole for this nest was obscured by vegetation therefore sentry ants were not counted; [2] These ants were collected and therefore not marked; [3] These ants were collected and therefore not marked; [4] These ants were not marked as they entered the nest; [5] These ants were usually already marked for a particular task and were easily observed, therefore they were not marked again unless their task changed.

The numbers of ants displaying these behaviours (according to their paint colour) were tallied at the beginning of every half hour (Figure 6-2). Additional ants not previously counted were marked with the appropriate colour and added to a cumulative tally of individuals displaying that behaviour for the half hour period. For example, at the beginning of each half hour all the ants on the nest surface that were marked yellow would be counted. If any additional ants were observed moving nest material during that half hour they were also marked yellow and added to the cumulative tally of ants. At the beginning of the next half hour, all the yellow marked ants were re-counted and any additional ants were marked and added to the tally. This process was repeated for behaviours observed during each half hour period.

Figure 6-2 Ant marked with craft paint to distinguish its behaviour.

If an ant was already marked blue ('exiting the nest') it was not re counted if it was subsequently found on the nest, as it could no longer be classified as a 'nest leaver'. These ants were re-marked with another colour and added to the tally for whichever task they were observed performing during that half hour period. Ants were only re-marked a maximum of once to reduce any stress caused by paint application.

If an ant had been re-marked, it was counted according to the behaviour it was displaying at that time e.g. if an ant was marked yellow and blue and within 0.5 m of nest, it was assumed that the ant was searching for nest material and therefore still involved in nest maintenance and not a forager. These assumptions were made based on previous observations of the ants at the nest, i.e. other individuals marked yellow would often search for nest material within 0.5m of the nest and then return to the nest surface.

6.2.2 Weather records

A weather station was used to record the microclimate conditions at the nest. The weather station instruments consisted of a Licor LI 200X pyranometer, HMP50 temperature and relative humidity sensor, 107 soil surface temperature sensor and CR10X data logger (Campbell Scientific). The weather station recorded the relative humidity (%) and ambient temperature (°C), in addition to the soil surface temperature (°C) and solar radiation (kW) at the nest surface (Figure 6-3). These recordings were taken every ten minutes and the average for every half hour period was calculated.

Figure 6-3 Photograph showing weather station components in situ.

6.3 Results

6.3.1 Spring

The greatest number of *M. pilosula* were observed on the nest surface between 0730 and 0800 (n=57) with a secondary peak between 1700 and 1730 (n=51) (Figure 6-4). Most of these ants were wandering on the nest surface (47.4%), others were exiting the nest (assumed to be foragers) (38.6%), involved in nest maintenance (8.8%) or within 0.5m of the nest (5.3%) (Figure 6-5a). The least number of ants were observed between 1130 and 1200 noon (n=3) (Figure 6-4a), and these were all involved in nest maintenance (Figure 6-5a). During each half hour period most ants were, on average, wandering on the nest surface (mean=11.9; S.E.=1.2, n=30), maintaining the nest (mean=7.2; S.E.= 0.8; n=30), or exiting the nest (mean=6.9; S.E.= 1.30, n=30).

6.3.2 Autumn

The greatest number of *M. pilosula* were observed on the nest surface between 0800 and 0830, also between 0900 and 0930 (n=27), with a secondary peak between 1800 and 1830 (n=13) (Figure 6-4). Most of these ants were exiting the nest in the morning (55.6% and 40.8% respectively) and returning to the nest without prey (69.2%) in the afternoon (Figure 6-5b). There were no ants observed between 1300

and 1330, also between 1400 and 1530, and after 1930. During each half hour period most ants were, on average, exiting the nest (mean=2.9; S.E.=0.9; n=26), returning to the nest without prey (mean=1.7; S.E.=0.5; n=26), or wandering on the nest surface (mean=1.6; S.E.=0.5; n=26). Out of all the ants observed wandering the nest surface (n=42) less than a quarter were observed subsequently exiting the nest (23%). The subsequent behaviour of the remaining ants (77%) was not observed.

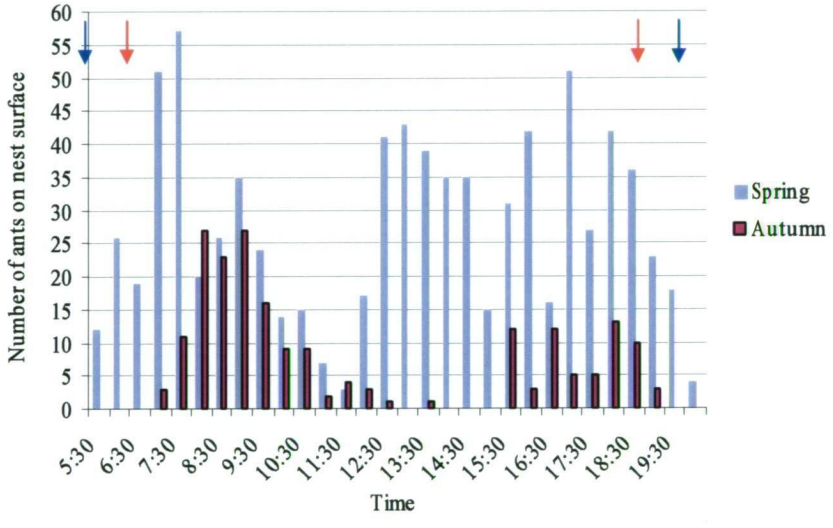
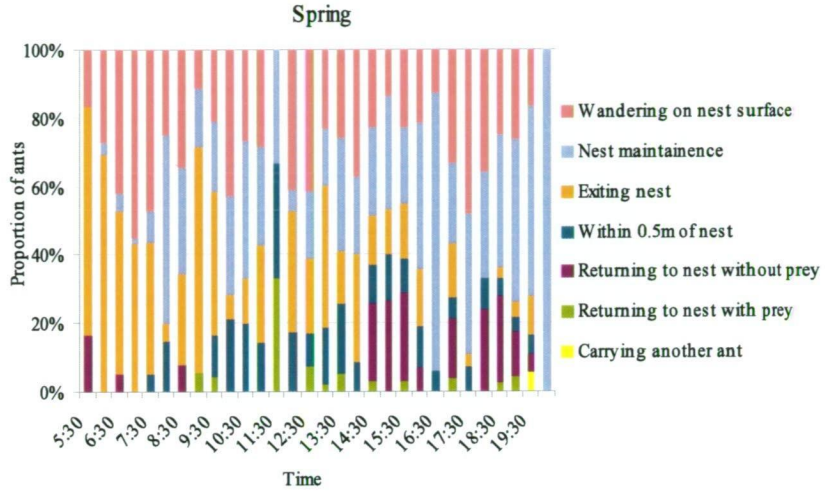
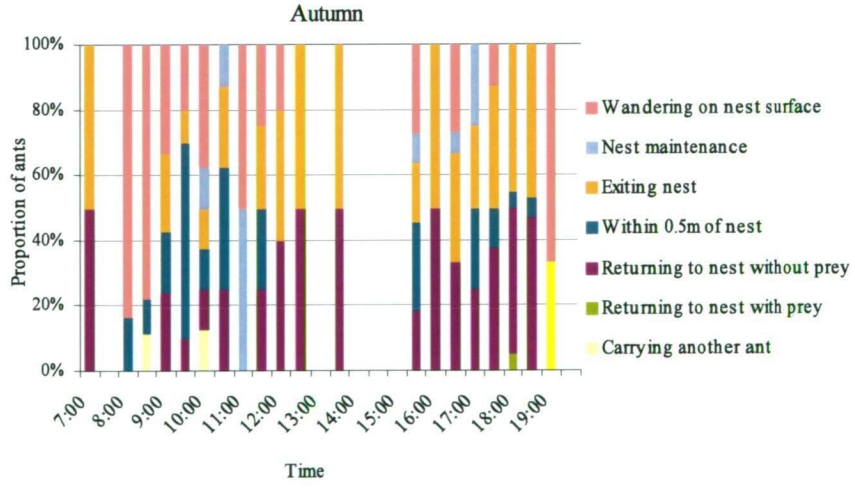


Figure 6-4 Number of *M. pilosula* on nest surface between sunrise and sunset in spring (blue arrows) and autumn (red arrows)



a)



b)

Figure 6-5 Proportion of *M. pilosula* displaying different behaviours throughout one day in a) spring and b) autumn

6.3.3 Wandering on nest surface

In spring, the highest number of ants wandering on the nest surface was observed between 0700 (n=28) and 0730 in the morning with a decrease in number to a minimum between 1130 and 1200 (n=3) and fluctuations in the afternoon until 1800 when the number of ants decreased steadily (Figure 6-6). The total number of ants observed wandering on the nest surface in spring was 245; of these, 27 subsequently became involved in nest maintenance and 41 eventually exited the nest. In autumn, the highest number of ants wandering on the nest surface were observed in the morning, between 0800 and 0830 (n=10). The numbers decreased until early afternoon between 1230 and 1500 when no ants were observed. Subsequently, numbers fluctuated in the late afternoon with no consistent presence of ants on the nest surface. In autumn, the total number observed wandering on the nest surface was 42; of these 10 were observed exiting the nest.

6.3.4 Nest Maintenance

In spring, nest maintenance occurred throughout the day except early in the morning (between 0530 and 0600). The highest number of ants maintaining the nest was between 1600 and 1630 (n=18). The peak period for nest maintenance was between 1600 and 1930 (Figure 6-6). During this time almost half the total numbers of ants (47%) on the nest, for each half hour period, over the entire day were maintaining the nest (Figure 6-5a). In autumn, only one ant was observed maintaining the nest in each of six half hour periods (between 1000 and 1130, 1530 and 1600, 1630 and 1700). No other ants were observed maintaining the nest at any other time of the day (Figure 6-5b).

6.3.5 Exiting nest

The highest number of ants left the nest in the morning in spring (between 0900 and 0930) (n=23) and autumn (between 0830 and 0900) (n=15) (Figure 6-6). In spring the first ants were observed leaving the nest between 0530 and 0600, the last was observed returning to the nest between 1930 and 2000. Over the duration of the day (0530 to 2000), 207 ants were observed leaving the nest. Of these, sixteen (8%) returned to the nest with prey, 62 returned to the nest without prey, 25 returned to the nest and became involved in nest maintenance, 10 returned to the nest and wandered

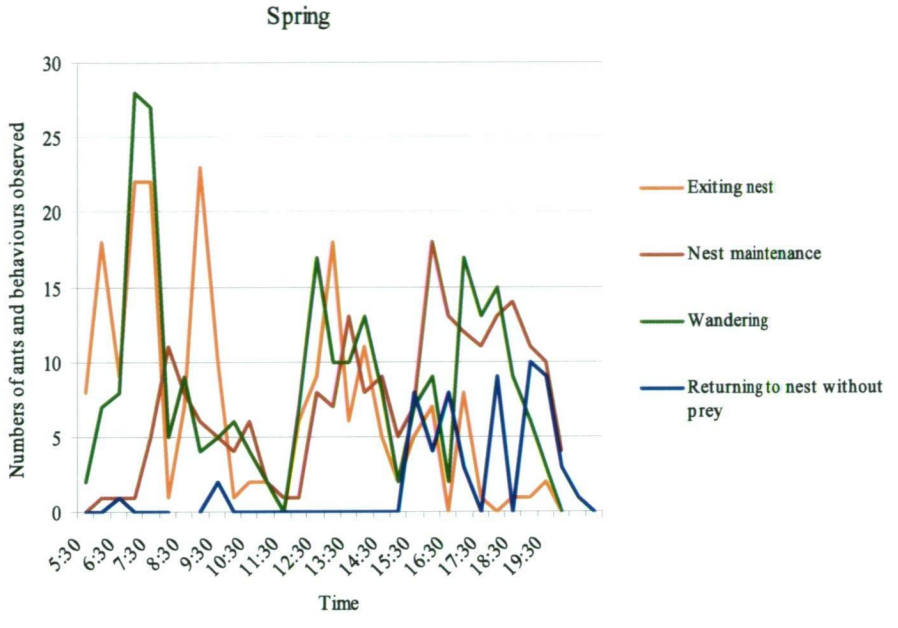
around and 94 were unaccounted for (Table 6-3). By 0800, 38% of the 207 ants had left the nest, 58% had exited by 1000. Out of all the ants that were observed leaving the nest after 0800 (n=127), none returned with prey. Only those that had left the nest prior to 0800 returned with prey. In autumn, the first ants were observed leaving the nest between 0730 and 0800, and the last was observed returning to the nest between 1800 and 1830. Over the duration of the day (0730 to 2000) a total of 77 ants were observed leaving the nest, of these, 84% had left by 1030. One returned to the nest with prey, one returned and became involved in nest maintenance and 31 were unaccounted for (Table 6-3).

6.3.6 Returning to nest

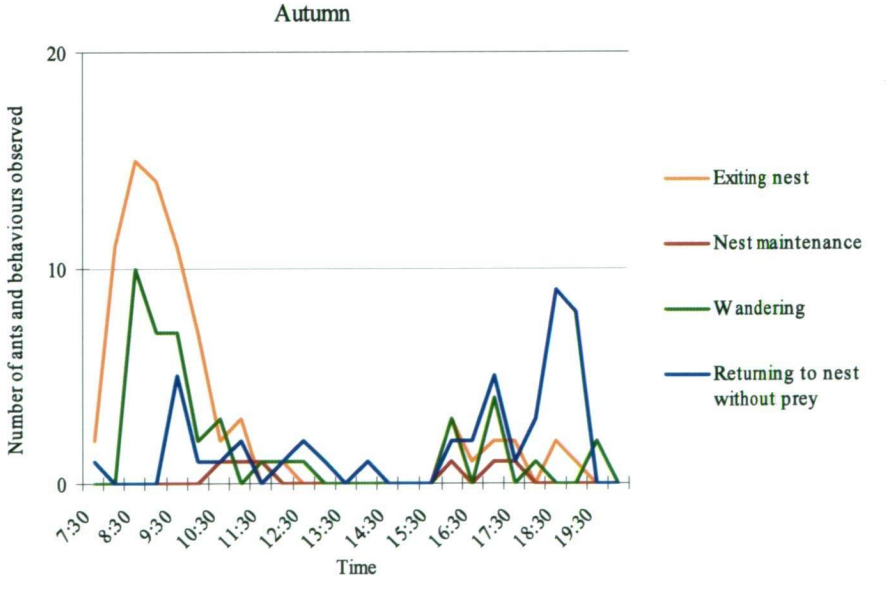
In both seasons the majority of ants returned to the nest in the afternoon. This pattern was most apparent in the spring with 94.8% of ants returning to the nest between 1430 and 2000. In the autumn, 66.6% of ants returned to the nest between 1530 and 1900 (Figure 6-6).

Behaviour	Number of ants	
	Spring	Autumn
Foragers (observed leaving the nest)	207	77
Returned to nest with prey	16	1
Returned to nest without prey	62	45
Returned to nest and became involved in nest maintenance	25	1
Returned to nest and wandered on nest surface	10	0
Number of *M. pilosula* that left the nest and were subsequently unaccounted for	94	31

Table 6-3 Number of ants that were observed leaving the nest on one day, and subsequent activities



a)



b)

Figure 6-6 Change in numbers of *M. pilosula* displaying the most observed behaviours in a) spring and b) autumn.

6.3.7 *M. pilosula* and prey

In spring, out of 113 *M. pilosula* that were observed returning to the nest, only 16 returned with prey (14.2%) and 11 of these were intercepted with a total of 19 prey items. The first ant with prey returned to the nest between 0900 and 0930, the last returned between 1900 and 1930 and most ants returned with prey in the afternoon (75%). The most common prey item was leaf beetle larvae (Chrysomelidae Paropsini A) (n=6). Most of the prey brought back to the nest were arboreal taxa (Table 6-4). In autumn, out of 77 ants that left the nest, only one ant returned to the nest with prey (1.3%) (Table 6-4).

Taxa	Number
Spring	
Syrphidae *Melangyna* larvae	3*
Bibionidae fly	1*
Culicidae fly	1*
Chrysomelidae Paropsini A larva	6*
Oecophoridae A larva	1*^
Oecophoridae B larva	1*^
Fergusoninidae fly	1*
Acrididae grasshopper juvenile	1*
Apidae *Apis* bee	1*
Chrysomelidae Paropsini B larva	1*
Gryllidae *Bobilla* (female)	1^
Insect larva cast skin (chrysomelid?)	1*
Total number	19
Autumn	
Erythraeidae	1*^

Table 6-4 Numbers and type of prey returned to nest in the spring. Prey items are described as ground dwelling (^), arboreal (*) or both (*^).

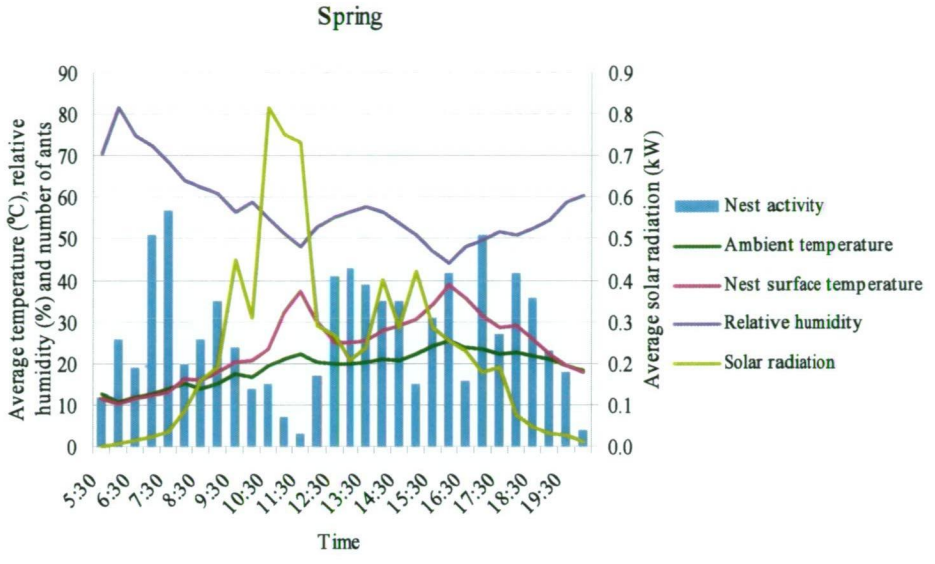
6.3.8 Weather Conditions

A summary of the microclimatic conditions recorded at the nest site is shown in Table 6-5.

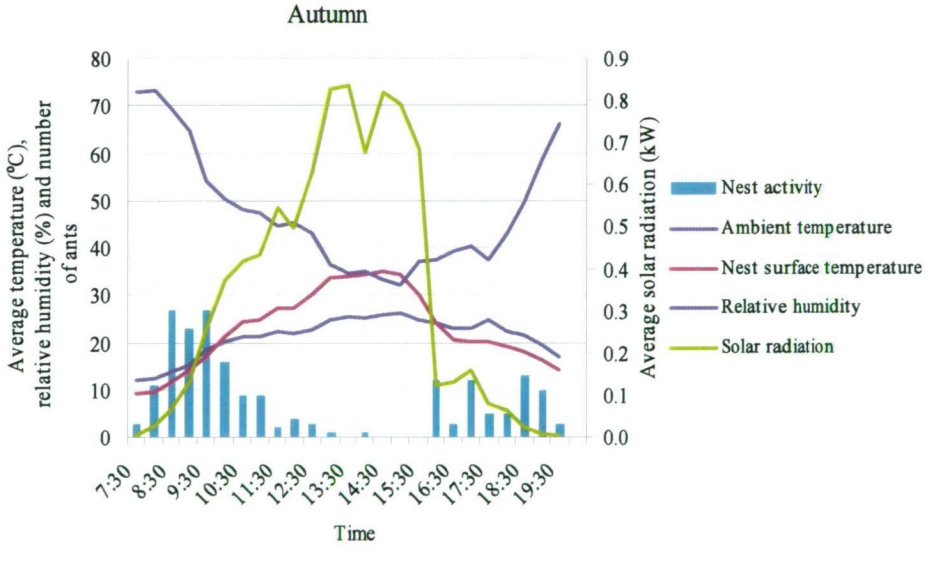
Season	Ambient temperature		Relative Humidity		Nest Surface temperature		Average solar radiation on nest surface (kW)
	Min.	Max.	Min.	Max.	Min.	Max.	
Spring	10.2	26.8	42.5	83.1	10.1	39.6	0.232
Autumn	11.9	26.6	32.2	74	8.9	35.2	0.349

Table 6-5 Climate conditions recorded by weather station at the nest site on a spring day and a similar autumn day.

The patterns of weather conditions throughout the day were similar between spring and autumn. Average half hourly nest surface temperature and ambient temperature increased and decreased at comparable times during the day. However, average nest surface temperature spiked twice in the spring while in autumn there was a gradual increase and decrease throughout the day. Ambient temperature increased and decreased gradually during these periods in both seasons. Relative humidity was highest in the morning then declined with slight fluctuations during the day before an increase in the late afternoon (Figure 6-7). In general, nest activity decreased during the periods that solar radiation and nest surface temperature increased (Figure 6-7).



a)



b)

Figure 6-7 Changes in total nest activity and weather conditions (half hourly averages) in a) spring and b) autumn.

6.3.9 Ant activity and weather conditions

There were no strong linear relationships between total activity and time in spring (r= 0.04). There were also generally no strong linear relationships between any of the weather factors and total activity, or any individual behaviour (Table 6-6).

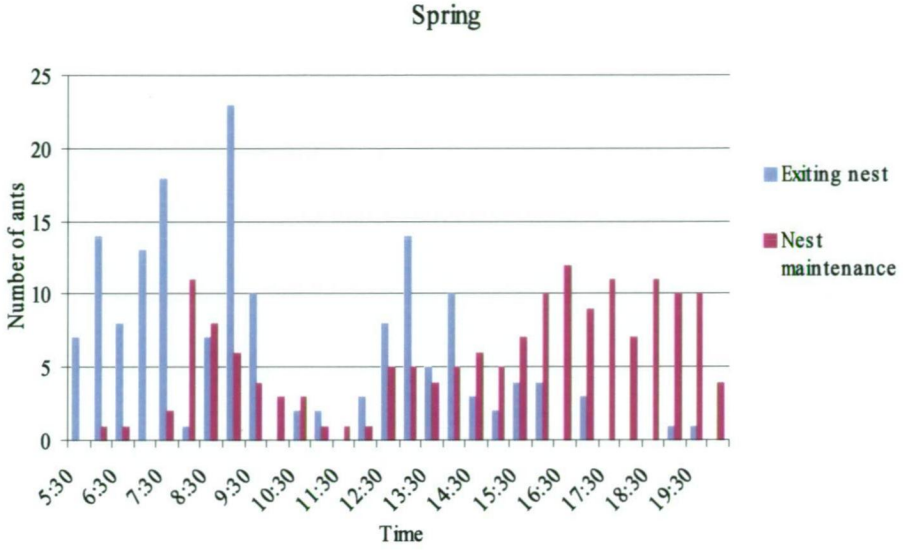
There was a negative linear relationship between total activity and time in autumn (r=-0.57). There was a negative relationship between some weather conditions and total activity in addition to some behaviours (Table 6-6).

Behaviour	Air temp	RH	SR kW	Nest surface temp
Spring				
Wandering	-0.23	0.27	-0.27	-0.23
Nest maintenance	0.45	-0.43	-0.23	0.27
Returning with prey	0.23	-0.21	0.11	0.17
Carrying another ant	-0.05	0.06	-0.18	-0.14
Entering nest	0.45	-0.39	-0.14	0.32
Exiting nest	-0.51	0.48	-0.08	-0.42
Within 0.5m	0.47	-0.40	0.28	0.45
Total activity	0.05	0.04	-0.22	-0.02
Autumn				
Wandering	-0.62*	0.41	-0.32	-0.43
Nest maintenance	0.21	-0.23	-0.1	0.05
Returning with prey	0.02	0.03	-0.21	-0.24
Carrying another ant	-0.3	0.34	-0.2	-0.24
Entering nest	-0.3	0.03	-0.41	-0.25
Exiting nest	-0.69*	0.64*	-0.42	-0.61*
Within 0.5m	-0.16	0.14	-0.20	-0.22
Total activity	-0.57*	0.54*	-0.52*	-0.61*

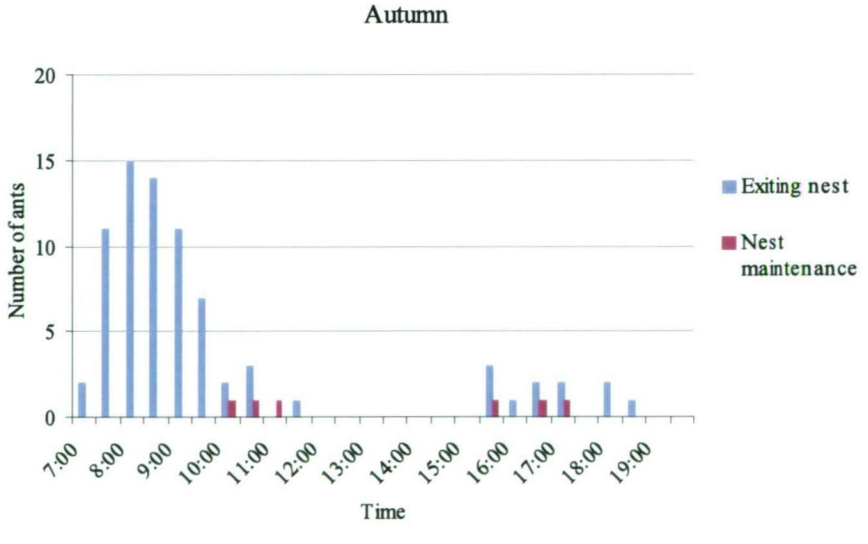
Table 6-6 Correlation co-efficients (r) showing generally low levels of linear relationship between behaviour and weather factors in spring; relationships between denoted with*.

6.3.10 Task priorities

While the numbers of ants exiting the nest was high in the morning, this activity decreased in the afternoon. The numbers of ants maintaining the nest show the opposite pattern with low numbers in the morning and an increase in the afternoon (Figure 6-8).



a)



b)

Figure 6-8 Numbers of ants leaving nest throughout day compared with the number engaged in nest maintenance

6.4 Discussion

Overall, patterns of activity were consistent between seasons. The most commonly observed behaviours were individuals exiting the nest and wandering on the nest surface. In spring nest maintenance occurred continuously throughout the day, whereas in autumn only limited nest maintenance occurred. A much greater proportion of ants returned to the nest with prey in spring than in autumn. Almost 50% of ants that were recorded leaving the nest, in both spring and autumn, were not observed returning to the nest. These ants may have returned the next day, died while foraging, been missed due to recording error, entered or joined another *M. pilosula* nest, or a combination of all of these.

6.4.1 Nest activity and weather

Results from this study suggest that the prevailing weather conditions did not affect the above ground nest activity of *M. pilosula* over the two observations days. However within days, there appears to be a modest negative relationship between ant activity and solar radiation in the middle of the day. The bimodal distribution of activity throughout the day in both seasons supports a non-linear relationship between solar radiation and activity levels. A general relationship between solar radiation and ant activity has previously been reported whereby activity changes as a thermo-regulatory reaction related to the shape and colour of the ant e.g. *Cataulacus guineensis* and *Myrmecia* sp (Ackonor 1984; Brown 1953). It is believed that some ants will naturally absorb more heat and compensate by decreasing activity when light intensity is high. Consequently the small size and dark colour of *M. pilosula*, further supports the theory that solar radiation may have an influence on its activity, which has not been fully revealed in this study. Further investigation into the relationship between *M. pilosula* activity and solar radiation is warranted.

6.4.2 Change in behaviours throughout the day

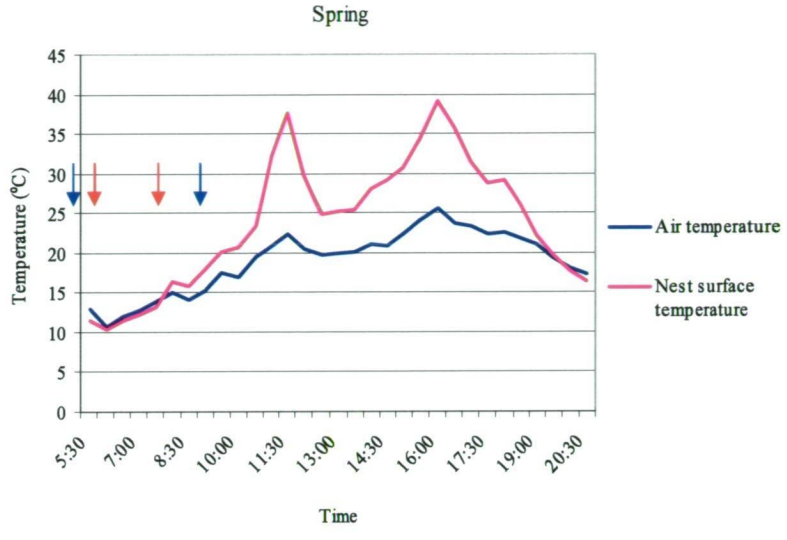
A possible change in the profile of behaviour of ants exiting the nest (in the morning) to ants involved in nest maintenance (in the afternoon) was observed. This pattern was most clearly seen in the spring results. A possible relationship between two behaviours throughout the day, suggests they are influenced by changing colony

priorities and are controlled by a mechanism not identified in this study. It is been demonstrated that in the ant *Pogonomyrmex occidentalis* the shifting of vegetation within the vicinity of the nest surface has thermoregulatory benefits (Bucy and Breed 2006). Therefore it could be possible that *M. pilosula* has shifted priorities during the day to assist with thermoregulation within the nest.

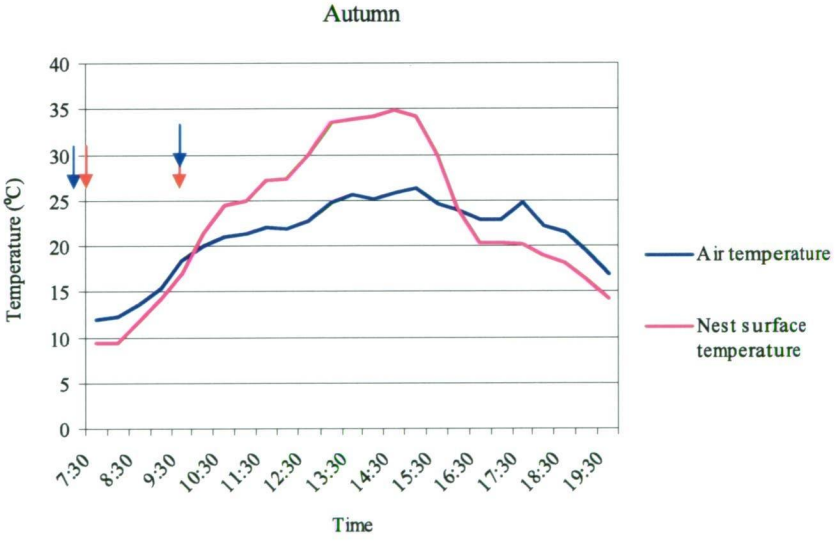
6.4.3 Wandering on nest surface

My results demonstrate that some of the ants observed wandering on the nest surface subsequently took on a task, either nest maintenance in the spring, or foraging. The influences behind this shift in behaviour are not clear. The fact that ants subsequently take on a task implies that they may receive environmental cues that guide their ensuing behaviour once on the nest surface. There was no evidence that the individuals were making an assessment of conditions to communicate to nest mates, or that nest mates on the surface were directing activity. As *M. pilosula* nest mates are not known to communicate via pheromones (Gray 1971b; Haskins and Haskins 1950; McLeman *et al.* 2002), it is possible that the individuals' behaviour is self regulatory.

The results also demonstrate that there are unlikely to be thermal benefits acquired from the wandering behaviour of ants on the nest surface as hypothesised in Chapter 5. Ants exited the nest during the coolest part of the day, before the nest surface temperature exceeded ambient air temperature, and before any benefits of radiant nest surface temperature could be acquired (Figure 6-9). Negative correlation co-efficients between wandering behaviour and temperatures support this likelihood. This indicates that the ants do not linger on the nest surface to warm up in preparation for other activities. Therefore this behaviour is more likely to be related to either spatial orientation or the assessment of external nest condition for maintenance purposes.



a)



b)

Figure 6-9 Graphs comparing ambient and nest surface temperature in a) spring and b) autumn. The blue arrows show the approximate time period when the number of ants exiting the nest was at its peak. The red arrows show the approximate time period when the number of ants wandering on the nest surface was at its peak.

6.4.4 Nest maintenance

As the material used to "decorate" the nest surface seems quite uniform in appearance it is reasonable to assume that the ants are very particular when gathering this material. It could be surmised that *M. pilosula* may choose nest sites and soil types conducive to its preferences for nest building materials. I found that individuals within 0.5m of the nest surface were involved in nest maintenance and appeared to be looking for nest material. I only observed one individual that strayed further than 0.5m from the nest to collect material and I did not observe any nest surface material being retrieved from within the nest. The meat ant, *Iridomyrmex purpureus*, has a very similar nest surface structure and also only collects material for the nest surface from the adjacent above ground habitat (Cowan 1985). Therefore it would be useful to determine whether the availability of nest surface material is considered at the time the colony founding, and how far the ants will search for nest material during this time. This information could be used to predict attractive nest sites.

6.4.5 Foraging activity

The difference in daily foraging activity between spring and autumn was reflected in arboreal sampling results (Chapter 5), where a greater number of *M. pilosula* were collected on foliage in the spring than the autumn, and a greater number of ants were observed exiting in the nest in spring than autumn. The type of prey intercepted at the nest was not indicative of invertebrates collected on vegetation associated with foraging *M. pilosula* (Chapter 5). Foragers demonstrated a low prey capture success rate. A few individuals were observed exiting the nest close to sunset, suggesting that some ants continue foraging into the night.

Some *Myrmecia* species are nocturnal foragers, or are more active overnight than during the day (Gray 1971b). Results from my study show that it is likely that *M. pilosula* forage into the night. This habit of foraging into evening has also been observed in *M. croslandi*, a species within the *M. pilosula* complex (Greiner 2007), and may change depending on the season (Gray 1971b). The timing of ants return from foraging has been examined in four sympatric species of *Myrmecia* and it was

determined that their periods of foraging activity reflected in different light conditions and were correlated with eye structure (Greiner 2007).

The bimodal activity pattern of ants exiting the nest, with the highest number of ants exiting in the morning has been observed in other types of ants, including other species of *Myrmecia* (Curtis 1985; Greiner 2007; Narendra *et al.* 2010). One hypothesis is that the light conditions in the morning are more conducive to prey capture (Narendra *et al.* 2010). However, if light intensity in the morning assisted prey capture, then it is not clear why the ants would continue to leave the nest to forage any other time of the day.

The high proportion of foragers returning without prey in both seasons could indicate that *M. pilosula* are foraging for liquid food sources. Although *Myrmecia* are considered specialist predators they are known to collect nectar as food for mature castes, while invertebrates are collected for the immature stages (Gray 1971b; Haskins and Haskins 1950). The high number of ants intercepted with prey in the spring compared to autumn signifies that a higher number of immature stages are being produced at this time of the year (Freeland 1958).

In some cases ants returned to the nest carrying more than one prey item. This indicates that *M. pilosula* do not return immediately to the nest after initial prey capture, but either remain foraging, possibly on the same vegetation where were first successful, or opportunistically on their return to the nest. This behaviour and the logistics of capturing and handling two prey items have not been further examined.

This study has identified that *M. pilosula* have a preference for leaf beetle larvae (*Chrysomelidae Paropsini* A) larvae. This is not consistent with the vegetation beating results in Chapter 5 in which no leaf beetle larvae were collected on vegetation with *M. pilosula* present at the time, nor was any other prey item intercepted at the nest in this study. This indicates the possibility that *M. pilosula* were foraging on these trees to collect nectar rather than insect prey. Flowering times of the plant species identified in Chapter 5 would need to be investigated to confirm this theory.

Chapter 7 Synthesis and Conclusions

Other than what is held in the ANIC at the CSIRO (www.antsonline) the distribution of a native ant within Australia has never been mapped this thoroughly. Modelling the climatic envelope for ant species has previously been limited to invasive ants (Korzukhin *et al.* 2001; Steiner *et al.* 2008; Sutherst and Maywald 2005). Given the relative importance of *M. pilosula* to the human population the prediction of its current total range provided is an encouragement to researchers to begin more targeted surveys of *M. pilosula* and generate a more accurate description of its habitat.

The bioclimatic profile generated by BIOCLIM (Chapter 4) could be used in conjunction with the plant species associated with *M. pilosula* in Chapter 5 to develop a more succinct prediction of current distribution on a small scale. For example, in Tasmania vegetation layers identifying woodland vegetation associated with *M. pilosula* (Chapter 5) could be combined with the bioclimatic profile of known *M. pilosula* locations (Lindenmayer *et al.* 1991). The generated predicted distribution would be invaluable information for those that are highly allergic who have the option of managing their lifestyle accordingly i.e. by making an informed choice on where to live, or where to camp for the weekend, and decrease the risk of encountering *M. pilosula.*

The health implications of increasing urbanisation of bushland and how this impacts on the interactions between humans and ants need further consideration. This would shed light on the question of why Tasmanian human communities appear to be more susceptible to *M. pilosula* stings than those on the mainland. It is likely that communities are more prone to *M. pilosula* stings because the ant may be more abundant in Tasmania than on the mainland. It can be speculated that the Tasmanian human population has a higher exposure due to the typically small buffer zone between urban and bushland areas. This may have implications for town planning projects in outer suburbs that encroach on native bushland. In this context results from Chapter 5 should be taken into consideration where I have identified that a higher likelihood of *M. pilosula* can be associated with particular tree species. These

plants should be disregarded for landscaping projects e.g. playgrounds, parks and road sides, in an attempt to reduce interactions between *M. pilosula* and the public in urban areas.

From the success of arboreal sampling (in the spring), it is likely that *M. pilosula* climbs on abiotic structures (such as fences and clothes lines) that could be mistaken for a pathway that may eventually lead them higher up in the vegetation layer where it is more likely they will catch their target prey. Therefore contact with tree branches or leaves, or other above ground structures such as picnic tables, fences, railings etc. should be avoided because of the likelihood that *M. pilosula* are using them as foraging pathways. These structures and any similar should not be installed in back yards, or council landscaping projects if possible.

It appears from this study that solar radiation is the most useful tool for predicting day to day activity of *M. pilosula.* Considering the limiting affects of high summer temperature on *M. pilosula* distribution on a continental scale (Chapter 4) and the apparent constraining impact of solar radiation on activity at a colony level (Chapter 6), a strong case can be made for a relationship between solar radiation and *M. pilosula.* Greater resolution of this relationship could be determined by daily observations of nests across seasons. Nest observations need only be made during minimum and maximum periods of solar radiation during the day.

It seems that *M. pilosula* activity is generally not limited by available food resources in that nest activity in autumn was low compared to in spring (Chapter 6) even though some food resources were more abundant in the autumn (Chapter 5). These observations support the idea that seasonal resource needs within the nest, driven by brood production and subsequent protein requirements (Haskins and Haskins 1950), are more influential on seasonal above ground nest activity, rather than microclimate or resource availability. Further investigation into seasonal brood production within the nest would give greater insight into the cycle of nest activity throughout the year.

Invertebrate associations with *M. pilosula* from arboreal sampling (Chapter 5) and the types of prey intercepted at the nest (Chapter 6) showed disparities. None of the invertebrates sampled on foliage with *M. pilosula* (Chapter 5) were the same as those intercepted at the nest. This partly suggests that *M. pilosula* has a wide range of

prey preferences. Prey characteristics that appeal to them could be as simple as being soft bodied and arboreal. Controlled investigations into preferred prey type could be developed with laboratory colonies for ease of application and greater replication for less effort.

In the future researchers should be aware of the reasons for a lack of activity in a colony and choose those appropriate for the type of study required. As shown in Figure 5-7 factors other than nest size may need to be considered when making a judgement. Studies indicate that nests will be least active just after founding (Freeland 1958) and after the queens death (Haskins and Haskins 1980). 'Typical age polyethism' (Holldobler and Wilson 1990) is known to occur in *Myrmecia* sp. which means that nests that have been newly founded will have fewer foraging workers as the new workers only attend the brood and queens until further generations of adults have been produced (Freeland 1958). Typical nests of *M. vindex* become moribund after the death of the founding female although queens have been known to live for up to 6 years (Haskins and Haskins 1980). After this time the nest will gradually become inactive. In the case of *M. pilosula*, where multiple queens have been found in one nest (Haskins and Haskins 1950) the life expectancy of a colony is less predictable.

A reasonable case was made in Chapter 5 that a large proportion of nest activity was actually on the nest surface rather than away from the nest (in terms of foraging ants). However, this hypothesis was not supported by results in Chapter 6, which show clearly that a high proportion of ants exit the nest during the day compared to the number of ants that remain on the nest surface. This highlights one of the limitations of observing multiple nests over one day. Future studies should focus on a pre-determined highly active nest observed over several days, seasonally, or a number of active nests in close proximity which can be observed for short periods (i.e. a rotation of 10mins at each nest) e.g. Hoffman (1998). Although laborious, this approach would be invaluable for documenting nest activity seasonally, in association with solar radiation (to assist prediction of daily activity cycles) and brood production (as a potential indicator of seasonal activity).

Some common behaviours were identified in Chapter 5 which required further

observation to comprehend the function, as was attempted in Chapter 6. The observations of behaviours such as wandering on the nest surface have been further clarified in that some of these ants do engage in a more obviously functional behaviour. But the purpose of the wandering behaviour itself remains unknown. In consideration of the evidence that *M. pilosula* uses visual clues for foraging, it is highly likely that the behaviour is a function of visual stimulation.

In general, the ants were more active in the spring than the autumn which supports the publics' perception that the ants are less active during the cooler months (P. McQuillan personal communication). Consequently those with allergies should avoid bush walking or activity in the vicinity of a *M. pilosula* nest during the spring. Avoidance of particular trees such as *Acacia stricta* and *Leptospermum scoparium* (Chapter 5), or those that might host the invertebrates from particular taxa (e.g. Gnaphosidae, Salticidae or Chrysomelidae) (Chapter 5, Chapter 6) should also be considered.

On a daily basis, the ant nests are more active in the mornings, when most of the ants exit the nest to forage. Therefore if outdoor activity is going to take place in the vicinity of a nest or bushland that is likely to have *M. pilosula* inhabitants (in regions identified in Chapter 4 or vegetation identified in Chapter 5) it should be limited to early mornings before the nest becomes active, in the middle of the day when activity on the nest surface clearly decreases, or late afternoon when most of the ants have returned to nest. Mid morning and mid afternoon outdoor activity should be avoided if possible.

It appears that *M. pilosula* behaviour may be broadly predictable as weather conditions change seasonally. This is demonstrated by the change in foraging activity (Chapter 5) and levels of nest activity (Chapter 6) between spring and autumn. To further examine this behaviour and quantify the speculated lack of activity over the cooler months, a wider range weather conditions needs to be studied. As the weather in Tasmania can be highly inconsistent, these measurements might best be made under controlled conditions, with cultured ant colonies. Laboratory colonies of *Myrmecia sp.* have been previously been used to study general aspects of their behaviour (Freeland 1958; Gray 1971a; Haskins and Haskins

1950; Morrison 1983) and these studies should be referred to for guidance.

Although differences in seasonal activity have been observed in this study results could not be extrapolated to other seasons or locations. Behavioural and activity differences may occur between species of *M. pilosula* and at different locations. Even so, it may be useful for the Hobart community to have a picture of the ant displayed prominently at the entrance to walking trails, public playgrounds and parks with text giving advice on the best ways to avoid the ants as described in this thesis.

This study has resolved that *M. pilosula* has a predictable distribution well fitted within an easily measured climatic envelope, in addition to identifiable behaviours and activity times that can be practically utilised to manage exposure to the ant. Therefore, further studies are warranted to further elucidate associations between the key points of discussion identified in this study. This is of vital importance for the continued study of *M. pilosula* and management of interactions.

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Appendix 1

Description and codes used for the identification of all arboreal insect taxa collected

Insect description			Code
ACARINA	Anystidae		ACAAny
ACARINA	Bdellidae		ACABde
ACARINA	Mite	red velvet	ACAMitrv
ACARINA	Oribatida	winged	ACAOriw
ACARINA	Trombiculidae	stout red	ACATrosr
ARANEIDA	Amaurobiidae		ARAAma
ARANEIDA	Araneidae	small	ARAAras
ARANEIDA	Araneidae	*Araneus*	ARAAraA
ARANEIDA	Araneidae	*Arcys*	ARAAraAr
ARANEIDA	Araneidae	tiny b&w	ARAAratb
ARANEIDA	Araneidae	microspider	ARAAram
ARANEIDA	Gnaphosidae		ARAGna
ARANEIDA	Hahniidae		ARAHah
ARANEIDA	Linyphiidae	*Linyphiidae cf*	ARALinLc
ARANEIDA	Nicodamidae	*Nicodamus*	ARANicNi
ARANEIDA	Oxyopidae		ARAOxy
ARANEIDA	Philodromidae		ARAPhi
ARANEIDA	Salticidae	Myrmarachne black small	ARASalMy
ARANEIDA	Salticidae		ARASal
ARANEIDA	Salticidae	*Opsithoncus*	ARASalOp
ARANEIDA	Sparassidae	*Neosparassus*	ARASpaNe
ARANEIDA	Theridiidae		ARAThe
ARANEIDA	Theridiidae	Theridiidae green white	ARATheTw
ARANEIDA	Theridiidae	Theridiidae grey	ARATheTg
ARANEIDA	Theridiidae	*Phoroncidia*	ARAThePh
ARANEIDA	Thomisidae	*Diaea*	ARAThoDi
ARANEIDA	Thomisidae	*Diaea* green white	ARAThoDg
ARANEIDA	Thomisidae	*Sidymella*	ARAThoSi
BLATTODEA	Blattellidae		BLABla
COLEOPTERA	Brentidae	*Apion* brown	COLBreAb
COLEOPTERA	Brentidae	*Apion* silver	COLBreAs
COLEOPTERA	Cantharidae	*Chauliognathus tricolor*	COLCanCb
COLEOPTERA	Chrysomelidae	*Monolepta*	COLChrMo
COLEOPTERA	Chrysomelidae	*Calomela*	COLChrCa
COLEOPTERA	Chrysomelidae	Chrysophtharta bimaculata	COLChrCb
COLEOPTERA	Chrysomelidae	Chrysophtharta goldspots	COLChrCg
COLEOPTERA	Chrysomelidae	*Paropsine L*	COLChrPl

COLEOPTERA	Coccinellidae	*Rhyzobius alphabeticus*	COLCocRa
COLEOPTERA	Coccinellidae	Rhyzobius brown	COLCocRb
COLEOPTERA	Coccinellidae	*Rhyzobius 2color*	COLCocRc
COLEOPTERA	Coccinellidae	*Rhyzobius larva*	COLCocRl
COLEOPTERA	Curculionidae	B	COLCurB
COLEOPTERA	Curculionidae	A	COLCurA
COLEOPTERA	Curculionidae	*Baris*	COLCurBa
COLEOPTERA	Curculionidae	Brachycerinae small	COLCurBs
COLEOPTERA	Curculionidae	Gonipterus	COLCurGo
COLEOPTERA	Curculionidae	*Merimnetes* long antenna	COLCurMl
COLEOPTERA	Curculionidae	*Orthorhinus*	COLCurO
COLEOPTERA	Curculionidae	*Rhadinosomus*	COLCurRh
COLEOPTERA	Curculionidae	Tychiini	COLCurTy
COLEOPTERA	Dasytidae		COLDas
COLEOPTERA	Dermestidae	*Anthrenus*	COLDerAn
COLEOPTERA	Elateridae	*Parablax*	COLElaPa
COLEOPTERA	Latridiidae	*Corticaria*	COLLatCo
COLEOPTERA	Nitidulidae	Nitidulidae	COLNitNi
COLEOPTERA	Phalacridae		COLPha
COLEOPTERA	Scarabaeidae	Melolonthinae	COLScaMe
COLLEMBOLA	Brachystomellidae		COLBra
COLLEMBOLA	Entomobryidae	variegated	COLEntv
COLLEMBOLA	Sminthuridae	Sminthuridae grey	COLSmiSg
DIPTERA	Cecidomyidae		DIPCec
DIPTERA	Chironomidae		DIPChi
DIPTERA	Empididae		DIPEmp
DIPTERA	Sciaridae		DIPSci
HEMIPTERA	Acanthosomatidae	Immature	HEMAcai
HEMIPTERA	Aphididae		HEMAph
HEMIPTERA	Cicadellidae	Green wingless	HEMCicgw
HEMIPTERA	Cicadellidae		HEMCic
HEMIPTERA	Cicadellidae	stripy	HEMCics
HEMIPTERA	Cicadellidae	Ulopini	HEMCicU
HEMIPTERA	Coccidae		HEMCoc
HEMIPTERA	fulgoroid		HEMful
HEMIPTERA	fulgoroid b		HEMfulB
HEMIPTERA	Miridae green		HEMMirG
HEMIPTERA	Miridae stout		HEMMir
HEMIPTERA	Psyllidae		HEMPsy
HEMIPTERA	Tingidae		HEMTin
HYMENOPTERA	Bethylidae	Bethylidae	HYMBetB
HYMENOPTERA	Braconidae		HYMBra
HYMENOPTERA	Chalcidoid	microwasps	HYMCham
HYMENOPTERA	Formicidae	*Anonychmyrma*	HYMForA
HYMENOPTERA	Formicidae	*Camponotus black*	HYMForCb

HYMENOPTERA	Formicidae	*Iridomyrmex*	HYMForI
HYMENOPTERA	Formicidae	*Myrmecia pilosula*	HYMForMp
HYMENOPTERA	Formicidae	*Notoncus*	HYMForN
HYMENOPTERA	Formicidae	*Ochetellus*	HYMForO
HYMENOPTERA	Formicidae	*Prolasius* black	HYMForPb
HYMENOPTERA	Formicidae	*Polyrachis hexacantha*	HYMForPh
HYMENOPTERA	Formicidae	*Rhytidoponera male*	HYMForR
HYMENOPTERA	Torymidae	*Megastigmus* large	HYMTorM
LEPIDOPTERA	Arctiidae	Lithosiinae larva	LEPArcLl
LEPIDOPTERA	Epermeniidae	Epermenia larva	LEPEpeEl
LEPIDOPTERA	Gelechioidea	larva	LEPGell
LEPIDOPTERA	Geometridae	larva	LEPGeol
LEPIDOPTERA	Geometridae	Chlorocoma larva	LEPGeoCl
LEPIDOPTERA	Geometridae	Chlenias larva	LEPGeoCh
LEPIDOPTERA	Geometridae	Chloroclystis larva	LEPGeoCc
LEPIDOPTERA	Hypertrophidae	Thudaca larva	LEPHypTl
LEPIDOPTERA	Lasiocampidae	larva	LEPLasl
LEPIDOPTERA	Lymantriidae	larva	LEPLyml
LEPIDOPTERA	Oenosandridae	larva	LEPOenl
LEPIDOPTERA	Zygaenidae	Pollanisus larva	LEPZygPl
MYRIAPODA	millipede		MYRmil
NEUROPTERA	Coniopterygidae		NEUCon
NEUROPTERA	Hemerobiidae	larva	NEUHeml
PSOCOPTERA	Psocoptera	wingless	PSOPsowl
PSOCOPTERA	Psocoptera	winged	PSOPsow
THYSANOPTERA	Phlaeothripidae	tube-tailed thrip	THYPhlt
THYSANOPTERA	Thripidae	yellow	THYThry